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EFFECT OF CERTAIN CALCIUM COMPOUNDS AND OTHER SUBSTANCES ON THE YIELD AND CALCIUM CONTENT OF SOME CROPS[1]

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Kentucky Agricultural Experiment Station

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HISTORICAL

In a former publication (7), the writer has shown by an improved method of analysis that many Kentucky soils have a very low total calcium content; in fact, from work done on several of the same samples, some of which has been published (6, p. 267–306) the calcium deficiency in many of our soils assumes equal importance with their low phosphorus and nitrogen content when the relative amounts of these elements removed by ordinary crops are considered.

At present, enormous quantities of limestone are being used on our soils to increase production. Very little significance has been attached, however, to the fact that in applying this material an essential element for plant growth is added and one which is removed in comparatively large quantities by crops.

It has been assumed heretofore that soils generally contain abundant calcium compounds to furnish an ample supply of this element for crop requirements. As soil-survey work has progressed, however, the accumulated data show that there are certain types in which the small percentage of total calcium found would indicate that there may be a deficiency of this element for permanent fertility. As a result, recent investigators and agricultural writers, for example, William Frear,[3] Halligan (2, p.21), Hopkins (3, p. 38 and succeeding reports), Thorne (8, p. 57), Van Slyke (9, p. 21), and Voorhees (10, p. 3) emphasize its importance and class it as one of the four probable limiting elements the others being nitrogen, phosphorus, and potassium. The assumption that calcium is of minor importance in most soils probably accounts for the fact that very few experiments have been carried on to determine its effect as a plant food element.

McIntire and Willis (4) described some experiments in which a comparison was made of the carbonates and silicates of calcium and magnesium on the growth of red clover in pot experiments, employing two types of soil. They

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to express his appreciation of the helpful suggestions offered by Dr. A. M. Peter, Head of the Department of Chemistry, during the progress of the work.

[3] In a personal letter to the author.

obtained better results with calcium silicate than with the carbonate. In their experiments, equal amounts of calcium were compared in the two materials, the silicate used being the native mineral, wollastonite. These authors also quote experiments made by Mieth (5) in which this investigator concludes that the easily decomposable calcium silicate should receive consideration as a source of lime for growing plants.

EXPERIMENTAL WORK

In view of the fact that many of our soils have been found to have a relatively low total calcium content, the experiments below were intended to determine, if possible, the effect of this element as plant food on the growth of certain plants in such soils.

It is very difficult to test the influence of calcium as an essential element of plants by adding a compound to the soil in which they are to be grown, because this brings into the problem the effect of the other elements contained in the same directly upon the plant as well as the effect of the compound upon certain properties of the soil, such as acidity and texture which influence plant growth. The plan adopted for these experiments was to compare the effect of adding calcium silicate, calcium citrate, or calcium oxalate, respectively, with that of adding magnesium silicate, silica or dextrin, carrying equivalent amounts of magnesium, silicon, or carbon.

The principal comparison was that of the calcium and magnesium silicates in equivalent amounts. It was assumed that the secondary effect of the latter compound upon the acidity of the soil would about equal that of the former, giving opportunity for the calcium to show its effect as plant food. The use of magnesium silicate to check the neutralizing effect of the calcium silicate was considered preferable to the use of either calcium or magnesium carbonate.

The silica treatments were carried on as a control on the silica contained in the calcium silicate, consequently the amount used exceeds that contained in the magnesium silicate. It may therefore be used to check both. The dextrin was used as a control on the carbon added in the calcium citrate and, incidentally, that in the calcium oxalate. The quantity of carbon added in the dextrin applied therefore equals that of the former and is about double that of the latter compound.

The materials were stock precipitated chemicals which in most instances were further purified by washing with water. Analyses are given in table 1.

The soils used in the experiments were taken from areas which showed a low average calcium content. They do not, however, represent the soils with the lowest calcium content to be found in these areas, but, on the contrary, some are above the average. Partial analyses of the soils used and other data follow:

TABLE 1

Percentage composition of chemicals used in pot experiments

	CALCIUM SILICATE	MAGNESIUM SILICATE	SILICA	CALCIUM CITRATE	CALCIUM OXALATE	DEXTRIN
Ignition	10.82	23.20*	4.58	69.98	61.38	99.84
SiO_2	63.22	60.68	93.26		0.06	0.16†
NH_3ppt	0.52	0.86				
CaO	17.70	0.70		29.76	38.80	0.02
MgO	0.43	13.11			0.11	
Na_2O	7.70		1.26		0.59	
K_2O	0.09		0.06		0.03	
Mn_3O_4					0.10	
Approximate formula‡	$2CaO, Na_2O, 7SiO_3, 4H_2O$	$MgO, 3SiO_2, H_2O$		$Ca_3C_{12}H_{10}O_{14}, 4H_2O$	CaC_2O_4, H_2O	

* Moisture.

† Ash.

‡ Calculated from analysis.

Soil no. 56583, Bath County, in the Devonian area. Surface soil from the farm of W. W. Penix, about 1½ miles southwest of Olympia, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1180	86	
Calcium	4473	1080	700
Limestone requirement	5828		

Soil no. 56584, Russell County, in the Keokuk-Waverly area. Surface soil from the farm of J. S. Dickenson, about 5 miles southwest of Dunnville, Ky., on the Dunnville and Jamestown Pike. Commercial fertilizers have been used.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	440	32	
Calcium	2720	1160	380
Limestone requirement	100		

Soil no. 56585, Floyd County, in the Eastern Coal Field area. Surface soil from the farm of Burr Hereford, near Cliff, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1580	36	
Calcium	1420	1580	560
Limestone requirement	2366		

Soil no. 56586, Laurel County, in the Eastern Coal Field area. Surface soil from the control plots of the Station experiment field near Fariston, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1100	24	
Calcium	3500	1260	320
Limestone requirement	774		

Soil no. 56587, Hardin County, in the St. Louis-Chester area. Surface soil from the farm of Harry Gatton, 3 miles northwest of Glendale, on the Bacon Creek Pike. Commercial fertilizers have been used.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	820	26	
Calcium	3140	1240	220
Limestone requirement	414		

Soil no. 56588, Taylor County, in the St. Louis-Chester area. Surface soil from the farm of S. B. Coppock, 1 mile south of Burdick, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	780	38	
Calcium	3580	2040	480
Limestone requirement	107		

Each soil was thoroughly mixed, put through a coarse sieve, and air-dried. The experiments were carried on in the greenhouse in 2-gallon stone jars supplied with drainage and 15 pounds of soil was used in each jar. Triplicate treatments were given in all instances, and equal amounts of distilled water were applied.

In the experiments below it is assumed that an acre of the surface soil, 6 inches in depth, weights 2,000,000 pounds. The treatments given were as follows:

Controls. Di-potassium phosphate was added to these and other pots of the series as described later.

Magnesium silicate. An amount carrying 607 pounds magnesium and 2186 pounds silicon per 2,000,000 pounds.

Calcium silicate. An amount carrying 1000 pounds calcium and 2346 pounds silicon per 2,000,000 pounds.

Silica. An amount carrying 2346 pounds silicon per 2,000,000 pounds.

Calcium citrate. An amount carrying 1000 pounds calcium and 1198 pounds carbon per 2,000,000 pounds.

Calcium oxalate. An amount carrying 1000 pounds calcium and 598 pounds carbon per 2,000,000 pounds.

Dextrin. An amount carrying 1198 pounds carbon per 2,000,000 pounds.

Each of these chemicals was put through 100-mesh sieve and thoroughly mixed with the soil in each instance. An application of a solution of di-potassium phosphate at the rate of 100 parts per 2,000,000 of soil was made to each pot at the beginning of the experiment.

The crops grown in rotation were soybeans, sweet clover, alfalfa, and oats. The soils were inoculated before planting each legume, and between crops they were stirred to a depth of 6 inches and pulverized. The plants were thinned to uniform size and distribution, and the number allowed to each pot was 5 for the soybeans, sweet clover, and alfalfa and 10 for the oats. Repeated cuttings of the sweet clover and alfalfa near the crown were made.

At harvest, the plants were cut close to the ground, the roots disregarded, and the air-dried weights were obtained. Composite samples were made of the plants and separately of the seed obtained from the triplicate pots in each treatment for the laboratory work. The samples were finely ground, after which ash and calcium determinations were made on them. The calcium was determined volumetrically by practically the same procedure followed in the improved method on soils mentioned above (7).

In order to test the effect of the silicates and other treatments on the lime requirement of the soils, acidity determinations by the Hopkins method (1)

were made before and after the materials were applied and after the crops had been grown. These results together with the weights of the crops from the three pots of each treatment and other data are given in tables 2–12.

TABLE 2

Yield of soybeans (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	19.76	2.07	19.00	2.45	16.75	0.53	26.03	0.89	27.16	1.53	17.89	1.45
Magnesium silicate	20.18	2.38	20.15	3.63	15.28	2.19	28.53	0.76	33.23	0.87	20.97	0.27
Calcium silicate	22.02	3.45	22.34	1.92	20.32	0.85	32.92	0.40	33.34	0.18	25.75	0.52
Silica	17.94	1.79	18.16	3.15	18.60	0.87	27.64	0.67	28.17	0.63	25.35	0.29
Calcium citrate	18.28	2.44	18.77	1.51	15.69	1.38	23.26	2.04	25.91	1.34	24.65	0.75
Calcium oxalate	19.54	1.55	20.13	2.84	17.44	1.19	30.34	1.19	30.11	1.15	25.52	1.33
Dextrin	19.84	0.74	15.51	0.97	11.29	1.14	24.02	1.27	18.22	1.74	20.18	1.51

TABLE 3

Yield of sweet clover and alfalfa (air-dry)

TREATMENT	SWEET CLOVER			ALFALFA		
	Bath County	Russell County	Floyd County	Laurel County	Hardin County	Taylor County
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	(No growth obtained)	17.27	9.53	19.85	20.69	21.12
Magnesium silicate	4.77	34.44	16.17	28.38	32.90	23.58
Calcium silicate	13.48	36.39	18.83	32.56	44.22	32.64
Silica	(No growth obtained)	13.14	8.44	13.00	20.83	22.30
Calcium citrate	8.79	35.75	16.36	28.64	35.04	30.08
Calcium oxalate	11.74	29.94	12.82	31.20	32.25	30.64
Dextrin	(No growth obtained)	16.08	8.14	19.73	24.33	18.93

DISCUSSION OF RESULTS

The yields shows that some of the soils responded favorably to the application of calcium compounds, but the increases obtained were more pronounced with some crops and treatments than with others, as shown below.

Soybeans. The highest yield of hay on each soil was obtained by the use of calcium silicate, but on only one soil did this treatment give the highest yield of seed, the remainder being lower than some of the controls comprising the magnesium silicate, silica, and control plots. The calcium citrate showed an increase in hay on one and in seed on three soils, while calcium oxalate gave increases in hay on five and in seed on two soils, compared with the dextrin and control soils. The yields of seed are so small and irregular in some instances that they are insignificant.

Sweet clover and alfalfa. All calcium treatments showed material gains on all soils. The results obtained with sweet clover on the Bath County soil are

TABLE 4

Yield of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	49.79	13.79	30.98	10.32	27.55	8.44	25.83	7.55	26.79	8.56	28.17	7.50
Magnesium silicate	40.58	7.17	47.41	14.97	38.04	10.29	29.88	8.63	41.61	13.64	52.10	12.86
Calcium silicate	40.19	11.95	55.43	19.41	35.98	10.42	31.18	10.44	47.96	21.56	41.69	14.28
Silica	44.98	16.03	30.29	9.66	30.43	10.09	27.48	8.65	26.35	9.21	25.58	6.86
Calcium citrate	24.78	6.19	40.67	13.04	35.35	11.23	32.59	12.22	41.92	15.80	38.24	10.84
Calcium oxalate	26.01	5.93	39.23	15.30	26.19	7.28	30.82	11.72	31.97	12.16	35.18	11.46
Dextrin	44.65	15.24	30.50	10.49	28.93	9.42	29.59	9.56	28.11	10.81	34.34	10.43

of interest since no growth was obtained in three of the four control treatments. Incidentally this soil showed the highest acidity. With some treatments the growth of sweet clover was nearly inversely proportional to the acidity found, but this was not always true.

Oats. Calcium silicate made gains in the straw on three and in the grain on five soils. Calcium citrate shows gains in both grain and straw on five soils and calcium oxalate on four soils, compared with the controls.

The good results obtained with magnesium silicate are interesting and cannot be entirely attributed to the neutralization of the acidity by this material. It is thought that part of the beneficial effect is due to the magnesium, as

TABLE 5

Ash content of soybean hay (air-dry)

TREATMENT	BATH COUNTY	RUSSELL COUNTY	FLOYD COUNTY	LAUREL COUNTY	HARDIN COUNTY	TAYLOR COUNTY	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	6.15	6.07	5.65	7.32	10.76	10.84	7.80
Magnesium silicate	7.37	6.94	6.42	8.45	8.96	11.39	8.26
Calcium silicate	8.02	7.54	6.74	8.99	9.62	10.59	8.58
Silica	6.97	6.96	5.47	7.45	9.55	10.52	7.82
Calcium citrate	6.70	6.57	5.67	6.91	9.73	10.20	7.63
Calcium oxalate	7.25	6.42	6.07	7.43	11.21	8.38	7.79
Dextrin	6.52	6.95	5.88	8.28	9.81	12.42	8.31

TABLE 6

Ash content of sweet clover and alfalfa hay (air-dry)

TREATMENT	SWEET CLOVER				ALFALFA			
	Bath County	Russell County	Floyd County	Average	Laurel County	Hardin County	Taylor County	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	*	7.80	10.09	8.95	7.20	8.38	6.86	7.48
Magnesium silicate	8.16	6.29	8.68	7.71	6.60	7.31	7.60	7.17
Calcium silicate	9.76	7.29	8.27	8.44	6.43	6.96	7.19	6.86
Silica	*	8.87	8.11	8.49	8.25	9.11	7.05	8.14
Calcium citrate	10.30	6.88	8.94	8.71	7.67	6.89	7.13	7.23
Calcium oxalate	8.92	6.89	11.41	9.07	7.10	8.37	6.42	7.30
Dextrin	*	8.13	8.51	8.32	8.02	7.81	7.76	7.86

* No growth obtained.

illustrated in some of the results obtained on the yield of seed. In this connection it might be mentioned that plant physiologists are agreed that magnesium bears some close relation to seed formation. Considering the amount applied, the fact that this material did not prove detrimental to growth is of interest since the calcium-magnesium ratio in the soils was materially changed by its addition. The beneficial effect obtained from the use of the magnesium silicate raises the question of possible deficiency of magnesium in soils, and it is the writer's intention to investigate this more fully.

TABLE 7

Ash content of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	5.40	3.10	4.87	2.82	7.33	3.66	5.62	3.30	6.16	3.46	6.72	3.79	6.02	3.36
Magnesium silicate	9.17	4.39	7.81	4.37	11.62	4.41	13.23	4.71	9.48	4.47	9.63	4.78	10.16	4.52
Calcium silicate	7.16	4.60	8.44	4.66	10.64	4.56	10.88	4.47	6.86	4.31	10.26	4.67	9.04	4.55
Silica	4.93	3.14	5.49	3.20	6.46	3.94	6.88	3.32	6.76	3.60	8.09	4.06	6.44	3.54
Calcium citrate	7.05	3.39	4.12	2.40	6.28	3.19	5.19	3.10	5.42	2.88	6.61	3.29	5.78	3.04
Calcium oxalate	7.16	3.85	4.06	2.30	7.07	3.61	5.44	2.97	5.86	3.28	5.93	3.22	5.92	3.21
Dextrin	5.79	3.09	5.52	2.73	6.26	3.45	5.46	3.04	6.89	2.94	6.29	3.27	6.04	3.09

TABLE 8

Calcium content of soybeans (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	1.16	0.141	1.82	0.135	1.50	Lost	2.30	0.179	1.99	0.157	2.35	0.169	1.85	0.156
Magnesium silicate	1.17	0.121	1.34	0.115	1.54	0.102	2.06	0.170	1.78	0.129	1.87	0.337	1.63	0.162
Calcium silicate	2.05	0.106	2.31	0.108	2.14	0.012	2.89	0.160	2.55	0.213	2.77	0.111	2.45	0.118
Silica	1.23	0.124	1.90	0.116	1.54	0.129	2.33	0.196	2.08	0.305	2.33	0.308	1.90	0.196
Calcium citrate	1.68	0.101	2.09	0.129	1.80	0.012	2.53	0.160	2.38	0.167	2.32	0.124	2.13	0.116
Calcium oxalate	1.76	0.124	2.30	0.093	1.96	0.080	2.72	0.158	2.26	0.158	2.45	0.125	2.24	0.123
Dextrin	1.00	0.144	1.97	0.154	1.48	0.021	2.43	0.159	2.01	0.181	2.29	0.221	1.86	0.147

Calcium silicate increased the average ash content of the soybean hay and oat grain, but neither this nor the other calcium treatments increased the ash of the other crops. Calcium silicate also increased the average percentage of calcium in the soybean hay, and the oat grain and the other calcium treatments increased the amount of this element in the soybean hay and in both the oat straw and grain. Calcium oxalate seems to have raised the calcium content of the alfalfa.

It is of interest to observe in table 12 that the relative acidities of the six soils remain the same after each treatment, the order beginning with the lowest acidity being Russell, Taylor, Hardin, Laurel, Floyd, and Bath. The only deviations from this order are in the silica series where Russell is larger than Taylor and in the calcium oxalate series where Hardin and Laurel test equal in acidity. The effect of the different treatments, however, is much more pronounced in some soils than in others, and it is difficult to explain the behavior of the same material in its influence on the acidity of different soils.

TABLE 9

Calcium content of sweet clover and alfalfa (air-dry)

TREATMENT	BATH COUNTY	RUSSELL COUNTY	FLOYD COUNTY	AVERAGE	LAUREL COUNTY	HARDIN COUNTY	TAYLOR COUNTY	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	*	2.08	1.98	2.03	1.65	1.46	1.62	1.58
Magnesium silicate	1.06	1.17	1.35	1.19	1.03	1.01	1.40	1.15
Calcium silicate	1.52	1.84	1.79	1.72	1.39	1.28	1.43	1.37
Silica	*	2.44	1.72	2.08	1.55	1.38	1.57	1.50
Calcium citrate	1.83	2.02	1.94	1.93	1.52	1.49	1.75	1.59
Calcium oxalate	1.67	2.05	2.42	2.05	1.58	1.66	1.84	1.69
Dextrin	*	2.22	1.38	1.80	1.48	1.33	1.82	1.54

* No growth obtained.

When the results of the pot tests are considered, there is some evidence which shows that the calcium in the different treatments has probably functioned as plant food in some instances and, furthermore, has exerted an influence on the ash and calcium content of the plant. Comparison of the averages of the calcium treatments with those of the no-calcium treatments in table 11 shows that more of this element was removed by the crops from the former than by that from the latter.

If there had been an adequate supply of available calcium present in the soil it would seem that some of these differences should not be so large especially where magnesium silicate was applied, since this substance materially reduced the acidity of the soil.

Many of our soils have a lower total calcium content than those used in these experiments. Taking into consideration that an application of 1 ton of limestone or of calcium phosphate per acre to some of our poor soils adds about as much calcium as is already present, there can hardly be any doubt that any in-

TABLE 10

Calcium content of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	0.374	0.086	0.564	0.105	0.423	0.096	0.497	0.152	0.450	0.115	0.455	0.117	0.461	0.112
Magnesium silicate	0.302	0.074	0.386	0.089	0.338	0.090	0.372	0.113	0.363	0.103	0.355	0.117	0.353	0.098
Calcium silicate	0.349	0.094	0.469	0.146	0.507	0.107	0.546	0.128	0.396	0.126	0.405	0.129	0.445	0.122
Silica	0.355	0.105	0.514	0.115	0.388	0.083	0.458	0.110	0.397	0.103	0.497	0.126	0.435	0.107
Calcium citrate	0.470	0.097	0.795	0.139	0.490	0.114	0.649	0.150	0.511	0.116	0.661	0.140	0.596	0.126
Calcium oxalate	0.511	0.133	0.802	0.153	0.631	0.123	0.757	0.145	0.679	0.134	0.590	0.116	0.662	0.134
Dextrin	0.412	0.104	0.659	0.119	0.414	0.094	0.410	0.117	0.461	0.077	0.401	0.104	0.460	0.103

TABLE 11

Calcium removed by crops; average for each group of three pots for each crop on all soils

TREATMENT	SOYBEANS, HAY AND SEED	SWEET CLOVER	ALFALFA	OATS, STRAW AND GRAIN
	gm.	*gm.*	*gm.*	*gm.*
Control	0.393	0.181	0.325	0.156
Magnesium silicate	0.379	0.220	0.325	0.158
Calcium silicate	0.641	0.394	0.500	0.205
Silica	0.433	0.150	0.281	0.145
Calcium citrate	0.451	0.392	0.497	0.227
Calcium oxalate	0.536	0.372	0.530	0.223
Dextrin	0.340	0.145	0.323	0.162
Average of calcium treatments	0.543	0.386	0.509	0.218
Average of no calcium treatments	0.386	0.174	0.314	0.155
Difference	0.157	0.212	0.195	0.063

TABLE 12

Effect of treatments and growth of plants on lime requirement

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Lime requirement‡	Relative acidity§	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*	
Control*	5828	100	100	100	2366	100	774	100	414	100	107	100
Control†	6281	108	71	71	1413	60	232	30	125	30	54	50
Magnesium silicate	4561	78	18	18	535	23	89	11	61	15	54	50
Calcium silicate	3690	63	11	11	107	5	46	6	39	9	36	34
Silica	5064	87	61	61	964	41	253	33	132	32	43	40
Calcium citrate	3073	53	18	18	221	9	57	7	54	13	29	27
Calcium oxalate	2884	49	18	18	111	5	43	6	43	10	25	23
Dextrin	4896	84	71	71	1453	61	278	36	143	35	64	60

* Soil used in experiments before any plants were grown and to which nothing has been added. Tested Dec. 7, 1918.

† Same soil after addition of 100 parts K_2HPO_4 per 2,000,000 and all crops were grown. Tested Aug. 28, 1920.

‡ Calcium carbonate required per acre to neutralize acidity.

§ Original soil = 100.

creased growth obtained as a result of applying these materials, or even of some commerical fertilizers to such soils, is due in part at least to the plant food calcium which they supply in addition to the other good effects which they may accomplish.

SUMMARY

1. Pot experiments in the greenhouse were made on six soils representing four different types in this State in an effort to determine if there was a deficiency of plant-food calcium.

2. Following applications of different calcium salts, four crops—namely, soybeans, sweet clover, alfalfa and oats—were grown in rotation. The effects of certain other substances were studied at the same time as a means of control.

3. In some instances the addition of calcium compounds to these soils increased the yield of some crops, both in grain and straw or hay.

4. The calcium treatments increased the ash and calcium content of some of the crops.

5. A considerable variation in the percentages of ash and calcium of the same plant, both in grain and straw or hay, was found when grown on different soils or even on the same soil where different treatments were given.

6. Silica treatments appear to have exerted a favorable influence on the yield of soybeans and oats on some soils.

7. Appreciable gains were obtained by the use of magnesium silicate in many instances, especially on the yield of some grain. These may be attributed in part at least to the beneficial affects of the magnesium, although there was some reduction of the soil acidity by this treatment.

8. Where magnesium silicate was applied to the soil the average calcium content of the hay or straw of all crops grown and of the grain of oats was lower than that by any other treatment.

9. The acidity of the soils was materially reduced by some treatments, but it is not believed that all of the increased yields can be entirely attributed to this fact. The effect of the same treatment on the acidity of different soils produced some interesting results which are difficult to explain.

10. Comparison of the averages of calcium with no-calcium treatments shows that the crops of the former usually removed much larger quantities of this element from the soil. This was probably due to the fact that where no calcium threatment was given, the plants were in need of an adequate supply available calcium for growth.

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INFLUENCE OF SULFUR OXIDATION UPON GROWTH OF SOY BEANS AND ITS EFFECT ON BACTERIAL FLORA OF SOIL[1]

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The oxidation of sulfur in the soil, and the influence of sulfur upon bacterial activities and development has been studied by many investigators. Sulfur transformation in the soil has long been known, but only in recent years has the mechanism of it been more fully under consideration. Although no attempt is made to give a complete bibliography on the subject, some of the more conclusive studies reported are included in the following review of literature.

Boulanger and Dugardin (3), in trying to explain the fertilizing action of sulfur, found that the effect of sulfur on crop yields was more marked with unsterilized soil than with sterilized soil, due to the oxidation of sulfur by bacterial activities. Demolon (6) studying the fertilizer action of sulfur found that but little sulfur was oxidized in a sterilized garden soil, and reached the same conclusions. Bernhard (2) states that the beneficial effect of sulfur for the control of potato scab, was due to the disinfection of the soil by sulfur. Brown and Kellogg (5) showed that different soils have unlike "sulfofying powers" and some of the factors influencing the change of elemental sulfur to the sulfate form were of a biological nature. Lipman and his associates (11) have shown that elemental sulfur is oxidized by the proper bacteria. Later studies at the New Jersey Experiment Stations, have confirmed the earlier experiments. Martin (13) recently published results obtained with inoculated and uninoculated sulfur for the control of potato scab, which show that inoculated sulfur produced greater amounts of soil acidity then did uninoculated sulfur, and was superior for the control of potato scab.

There has been, and still is, considerable controversy as to the necessity of applying sulfur to the soil for the stimulation of plant growth. Lyon and Bizzel (12) report that in the lysimeter experiments at Cornell the sulfate sulfur in the drainage water was from three to six times as great as in the crops, while the sulfur content of the drainage water from the unplanted soil was about equal to the sulfur content of the crops and drainage water from the planted soil. Swanson and Miller (21) state that the loss of sulfur due to the amount taken up by crop is insignificant as compared with the total amount which has disappeared from the soil. Hart and Peterson (8) calculate the loss of sulfur in drainage water to be three times the amount brought down to an acre from the atmosphere. Stewart (20) concludes that under humid conditions sulfur need not be added to the soil as plant food. Suffi-

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cient sulfur is brought down by the rainfall to supply plant needs. Ames and Boltz (1) conclude that the cultivation of silt loam for 16 years without the addition of fertilizers has decreased the total sulfur supply.

Some striking results have been obtained with the use of sulfur as a fertilizer or stimulant. Reimer (17) obtained large increases in alfalfa yields by the use of elemental sulfur. A number of Oregon soils are deficient in sulfur and respond readily to sulfur applications as is shown by the work of Reimer and Tartar (18), who report increases of from 50 to 1000 per cent for alfalfa and clover by the use of fertilizers containing sulfur on various soil types. Flowers of sulfur produced beneficial results similar to those obtained by the use of superphosphate, gypsum, iron sulfate, etc. Headley (9, p. 16–18) reports experiments with sulfuric acid and gypsum and states that the yields of the soils so treated has been decidedly greater each year than those from the untreated plots, but not enough greater to be profitable. Miller (14) concludes from pot experiments that the addition of elemental sulfur enhanced the growth of the plants, and that the corresponding increases obtained on the soil extract indicated that sulfur acted directly in promoting this growth. Tottingham and Hart (23) made greenhouse and field tests with sulfur and composted sulfur and rock phosphate. They conclude that on two loam soils sulfur increased the growth of clover and cruciferae on one soil and not on the other. Sulfur increased the yields of barley seed on a silt loam apparently in need of lime. One hundred pounds of sulfur per acre was most effective.

The action of sulfur and the sulfates formed in the soil upon the soil bacteria and their activities has been studied in several instances. Janicaud (10) states that sulfur has a favorable influence on the development of bacteria in the soil. Duley (7) concludes that sulfur and gypsum increase the number of nodules to a marked extent on red clover roots in certain Missouri soils. Boulanger and Dugardin (3) studied the effect of sulfur on ammonification, nitrification, and nitrogen fixation and they found that the presence of small amounts of sulfur decidedly increased the activity of ammonifying bacteria. Brioux and Geurbet (4) challenged the conclusions of these investigators and say that sulfur as such does not have any influence upon the activities of ammonifying or nitrifying bacteria. O'Gara (15) reporting field experiments with field and truck crops in which elemental sulfur was added at a rate of 400 pounds per acre states that "one of the striking effects of sulfur treatment of soils on the soil microörganisms is the decided and consistent increase in total bacterial numbers as determined by the plate method." Tacheuchi (22) concludes that sulfur applied at the rate of 0.5 per cent and over decreases the number of bacteria in the soil, and that sulfur alone does not affect the root development, or the number of root nodules.

Miller (14) states that the great increase in the nitrogen content of clover grown on soil where sulfates had been added is the result, in all probability, of the sulfates stimulating the action of legume bacteria; the number of nodules on the clover roots were increased. Pitz (16) found elemental sulfur to increase soil acidity, and that the number of bacteria which would grow on agar plates decreased after a certain period, if sulfur was added to a silt loam soil. He reports an increase in ammonification, accompanied by a parallel decrease in nitrate formation.

EXPERIMENTS WITH SLIGHTLY ALKALINE SOILS

From the short review of literature it seems clear that some soils respond to the treatment of sulfur and others do not. The study reported below was primarily undertaken to determine whether or not a slightly alklaine soil, which produces chlorosis, would be benefited by additions of sulfur.

Determinations were made on numbers of the microörganisms in this soil, influence on the physical structure (flocculation, turbidity, water-holding capacity, change in volume), and the influence on the growth of soy beans.

The soil used was a Hanford fine sandy loam, and secured by Prof. C. F. Shaw of the University of California from ranches near Pomona, Cal. Professor Shaw wrote the following notes about these soils and their history:

These soils were secured from a point about two miles west of the business center of Pomona. Soil 1 was taken in an orange grove about 100 feet from the west line of the orchard. The orange trees were about thirty years old. They have been manured annually with barnyard manure, but the quantity applied was unknown by the owner. He has approximately seven acres in the grove and applies to it all the manure from one horse and one cow, spreading it on as it is made. The grove has been irrigated by furrows ever since it was first set out. The water has flowed from the east towards the west and the block of soil between each row has not received water except as it has run over from the shallow furrows or has seeped in by lateral movement. There has been slight accumulation of finer sediments in the west side of the grove—the side from which the soil was taken. The soil was taken from about the middle of the row between the trees and from the low ridge between two irrigation furrows. The land had been irrigated about one week previous. These samples represent a good soil in which the fertility has been maintained reasonably well through fertilizing and through tillage.

Soil 2 was taken about 150 feet west of soil 1 and about 50 feet west of the boundary line between the orange grove and the adjoining corn field. The field from which soil 2 was taken has been in corn or in barley annually for a great many years. It has been farmed nearly every year for thirty or more years, and no fertilizer has ever been applied. The ranch is run by a Mexican tenant and the system of culture is poor. During the last 6 years the field has grown at least 4 and possibly 5 crops of corn and one and possibly 2 crops of barley. At first the crop yields were good but they have dropped off during the last ten years. The field was in corn this year with a fair stand.

A mechanical analysis made of these soils showed that they were very fine. The data is given in table 1.

TABLE 1

Mechanical analyses of Hanford fine sandy loam

GRADE OF SOIL	ORANGE GROVE	OPEN FIELD
	per cent	*per cent*
Fine gravel	2.85	6.65
Coarse sand	9.99	9.34
Fine sand	11.02	11.21
Very fine sand	33.58	33.05
Silt and clay	42.56	38.91

Flowers of sulfur was inoculated with one per cent of soil which was known to contain sulfur-oxidizing organisms. This inoculated sulfur was then thoroughly mixed with soils 1 and 2 and the water-holding capacity determined. The soils were kept at 60 per cent of the water-holding capacity throughout the period of investigation.

Hydrogen-ion concentration determinations were made of the soil extract before and after mixing with sulfur and at definite intervals during the growth of the plants, using the apparatus described by Van Alstine (24).

The soils were divided into 2 parts, one to be used for plant cultures and the other for bacterial studies.

SERIES 1. VEGETATION EXPERIMENTS WITH SOIL 1

Soy beans were grown in earthenware pots filled with 5 kgm. of soil. Different amounts of inoculated sulfur were thoroughly mixed with the soil. To duplicates were added, besides the same quantities of inoculated sulfur, 300 pounds of rock phosphate per acre, and to triplicates, in the place of rock phosphate, 100 pounds of acid phosphate per acre. The exact amounts of sulfur added to all series were as follows:

CULTURE NUMBER	POUNDS PER ACRE
1, 6,11	None
2, 7,12	100
3, 8,13	300
4, 9,14	500
5,10,15	1000

The soy bean seeds were selected for size, germinated and transplanted when from 2 to 3 inches high, care being taken to select plantlets as nearly alike as possible. Six plants were planted in each pot and grown for 9 weeks. At the beginning of the experiment the soil was inoculated with a water extract of a soil containing soy-bean nodule-forming bacteria. The optimum water content of the soil was maintained by daily additions of distilled water, the pots being placed on the scale pan every 2 or 3 days. Notes were taken at intervals and the hydrogen-ion concentration of the soil extract determined once each week.

The influence of plant growth on the hydrogen-ion concentration was but slight. In the water extract of the soils treated with small amounts of sulfur the changes were not very marked, but the larger quantities of sulfur exerted a decided influence, although not as much as would be expected from the rather heavy applications. This was probably due to the slow oxidation of the sulfur.

Notes taken after 3 weeks show that, in general, the plants in soil to which sulfur was added were slightly behind the check cultures. Most of these plants started to develop small yellow spots on the leaves. According to the notes taken after 6 weeks, when 3 of the 6 plants were harvested, it appears that these yellow discolorations, which more or less resembled mosaic, were not found on the plants grown in soils which received 300, 500 and 1000 pounds of sulfur per acre, and that the plants grown in soil to which rock phosphate had also been added, had been very slightly affected, while the effect on the plants grown in the cultures receiving the same amounts of sulfur but acid phosphate instead of rock phosphate were more distinct. The yellow spots on the plants in the other cultures were at that time more pronounced than after 3 weeks. The soil used was at the beginning of the experiment slightly alkaline, or neutral, with pH values varying between 7.2 and 7.0.

A comparison made at the end of 6 weeks of all pot cultures seemed to place the plants receiving sulfur alone as best.

After 9 weeks the plants were scored again and the yellowish, sickly looking leaves counted. In all cases the yellowness increased with the increase of the quantities of sulfur employed. The plants grown in soil with sulfur alone seemed to be still ahead. At the end of 9 weeks the plants were harvested, and a comparison made on the relative numbers of nodules and on the extent of the root system.

It appeared that the check plants had all a more extensive root system than the plants grown in the sulfur-treated soils, with the exception of plants receiving 100 pounds of sulfur to the acre. On the latter the nodules were also more numerous than on the check plants and the plants treated with higher amounts of sulfur. The nodules decreased numerically with the increase of the amounts of sulfur applied. The plants which were grown in the soil receiving 1000 pounds of sulfur per acre had very few, but extremely large, nodules. Some of these nodules were $\frac{3}{4}$–1 cm. in diameter.

Although the weight of the plants decreased toward the highest sulfur application, the differences were not striking. It was concluded, therefore, to grow another crop of soy beans in the same pots with similar applications of sulfur, rock phosphate and acid phosphate.

The plants were selected and planted as before and 3 out of 6 plants harvested after 4 weeks, leaving the 3 best-looking plants in each pot. At this time the cotyledons of the check plants and of the plants receiving 1000 pounds of sulfur per acre were all still dark green, while most of the cotyledons of the plants receiving 100, 300 and 500 pounds of sulfur per acre, had been dropped previously or had turned yellow before the end of 4 weeks. The best looking plants were at that time in the check pots.

It was interesting to note that the pots receiving 1000 pounds of sulfur per acre and 300 pounds of rock phosphate in addition had a much higher hydrogen-ion concentration than the pots receiving acid phosphate in addition, and also higher than the pots which received 1000 pounds of sulfur alone. The hydrogen-ion concentration went up more or less in all cultures.

According to the notes taken, all plants were found blooming after $4\frac{1}{2}$ weeks, while cultures 5, 10 and 15 had very yellow leaves of which some had been dropped at that time.

Results are given in table 2.

The results obtained were much more pronounced than those obtained with the first crop. The fact that inoculated sulfur and rock phosphate together produced poorer results than sulfur alone can not fully be explained by supposing that this particular soil does not respond to phosphorus, since the results obtained with the acid phosphate treated pot cultures indicate that some benefit might have been derived from the phosphorus treatment. The cause for the poorer results seems to lie in the greater acidity produced.

The root systems of the plants receiving 100 pounds of sulfur were, in general, more extensive than those of the plants in the check pots, but the roots of the plants in pots with higher sulfur applications were considerably less extensive.

TABLE 2

Yields of tops of second crop of soy beans grown in orange grove soil for 6 weeks

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TRANSPIRATION AND EVAPORATION	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION	REMARKS
			Treated with sulfur					
	gm.		*cc.*	*cm.*		*pH*	*pH*	
1 (check)	3.0302	100.0	4150	26.5	10	6.9	6.6	
2	2.9453	97.3	4170	26.5	15	6.9	6.5	
3	2.1595	71.4	3710	21.0	2	6.6	6.3	
4	2.2741	75.1	3535	21.0	6	6.6	6.1	Slightly injured
5	2.0000	66.0	3305	21.5	5	6.2	6.0	Strongly injured, cotyledons green
			Sulfur plus 300 pounds of rock phosphate					
6 (check)	3.2750	100.0	3875	21.5	13	7.0	6.7	
7	2.9348	89.6	3710	22.0	12	6.9	6.4	
8	2.8441	86.9	3755	23.0	10	6.8	6.2	
9	2.1642	66.2	3390	22.0	7	6.5	6.1	
10	1.3470	41.1	3080	19.0	0	6.1	4.6	Strongly injured, cotyledons green
			Sulfur plus 100 pounds of acid phosphate					
11 (check)	3.0227	100.0	3695	24.0	8	7.0	6.7	
12	3.2712	108.1	4030	26.5	15	6.9	6.5	
13	2.5400	84.0	3480	23.0	7	6.8	6.3	
14	2.4882	82.5	3505	23.0	8	6.6	6.1	
15	1.9160	63.3	3235	21.5	3	6.2	5.8	Injured, green cotyledons

Inoculated sulfur applied in moderate quantities, therefore, might aid the plants to get hold of more plant food through stimulation of root development.

The number of nodules increased with the application of 100 pounds of sulfur but decreased considerably if greater quantities of sulfur were employed. The size of these nodules increased with the decrease of their numbers. The relative number of nodules for the different cultures, placing the checks at 100, were as follows:

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
1	100	6	100	11	100
2	120	7	150	12	120
3	100	8	100	13	120
4	80	9	80	14	100
5	20	10	10	15	60

The decrease in numbers of nodules for the plants receiving acid phosphate were not as great as for the plants without phosphate, and this can be attributed to the available phosphorus since the plant receiving rock phosphate decreased in numbers in the same manner as without phosphorus, and although the actual acidity of culture 5 was even much less than of culture 15, more nodules were produced in the latter. The numerical decrease of the nodules is undoubtedly due to the sulfate formation and the consequent increase of acidity.

VEGETATION EXPERIMENTS WITH SOIL 2

Similar experiments were made with soil 2, which was poorer in nitrogen and mineral food constituents. Soy beans were planted in the same manner as has been previously described, and sulfur, rock phosphate and acid phosphate employed at the same rates.

The changes in acidity were in this case quite similar to the changes in series I. After 3 weeks the plants were, in general, much poorer than the plants grown in series I. The leaves of nearly all plants had yellow spots, and some of them were more or less drooping. The general appearance and average height were considerably inferior after 6 weeks when 3 of the 6 plants from every culture were harvested, than was the case with plants grown in soil 1. The effect of sulfur was more pronounced and cultures receiving 1000 pounds of sulfur per acre were severely injured.

The soil responded far better to the treatment of phosphorus than the soil which had received fertilizers for the last 30 years. The cultures receiving rock phosphate produced, in general, higher yields than the cultures to which sulfur alone was added. In all cases again sulfur applications of 100 pounds to the acre gave slightly increased yields, while the others produced less dry weight.

All plants were scored for general appearance, yellowness and stockiness before harvesting. The scores show even more clearly than the dry weights of the plants that all cultures receiving 100 pounds of sulfur were better looking, greener and less spindling than the check plants or the plants grown in pots which received higher applications of sulfur.

A comparison of the nodules and root system brought out more definitely the same facts as were found in soil 1. The extent of the root system and also the number of nodules decreased with the increase of the sulfur applications, except on plants in cultures 2, 7, and 11, which were relatively greater.

Here again, a second crop of soy beans was planted in the same pots and all applications of sulfur, rock phosphate and acid phosphate were repeated.

The hydrogen-ion concentration in this second-crop experiment was considerably higher than in the case of the second crop on soil 1. The highest concentration was again found in culture 10, which received 300 pounds of rock phosphate to the acre besides 1000 pounds of sulfur. A number of the cultures were apparently less acid after 6 weeks than at the end of 5 weeks.

Notes taken a few days before harvesting show that the cotyledons of the cultures receiving 300 and 500 pounds of sulfur had in nearly all cases been dried out or dropped, while cultures receiving higher applications of sulfur had still dark green cotyledons. Culture numbers 3, 8 and 13 seemed most mature, while culture numbers 5, 10 and 15 were severely injured.

The dry weights and other data secured from the second crop are given in table 3.

TABLE 3

Yields of tops of second crop of soy beans grown in soil receiving no fertilizers for more than 30 years

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TOTAL TRANSPIRATION AND EVAPORATION	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION	REMARKS
			Treated with sulfur					
	gm.		*cc.*	*cm.*		*pH*	*pH*	
1	2.5990	100.0	3800	21.5	5	6.7	6.5	
2	2.4328	93.6	3500	21.6	5	6.7	6.2	
3	2.2447	86.5	3245	22.0	7	6.5	5.0	
4	1.9295	74.3	3110	19.0	5	6.0	4.9	
5	1.1808	45.4	2595	16.5	1	5.3	4.0	Two plants blooming, strongly injured
			Sulfur plus 300 pounds of rock phosphate					
6	2.3855	100.0	3665	22.0	9	6.9	6.3	
7	2.3700	99.5	3345	23.4	8	6.4	6.0	
8	2.1425	90.1	3435	21.5	4	6.3	6.0	
9	1.9261	80.8	2880	19.0	7	6.3	5.5	
10	1.1520	48.4	2715	13.5	2	6.1	3.8	Strongly injured
			Sulfur plus 100 pounds of acid phosphate					
11	2.2160	100.0	3395	21.5	10	6.7	6.4	
12	2.5500	114.6	3445	24.2	10	6.6	6.1	
13	2.1645	97.4	3330	22.4	8	6.6	6.0	
14	1.9410	87.3	3545	19.0	7	6.3	5.7	
15	1.9863	89.2	3320	18.5	5	6.3	4.5	Medium injured

It is apparent that plants grown in soil receiving acid phosphate in addition to sulfur were best. The whole series which received rock phosphate in addition to sulfur were poorer than the cultures receiving sulfur alone. The acidity produced in these pots did not seem to be enough to make the rock phosphate available, although some of the injury to the plants can be attributed to the acidity. Still this does not explain the phenomena in full. It is very likely, therefore, that the relatively high amounts of sulfates formed were harmful or prevented the plants from taking up the necessary food constituents. The results with this soil poor in nitrogen and mineral food constituents brings out clearly

the fact that the acidity produced was not able to supply greater quantities of the necessary plant food elements.

The nodule formation was also influenced by the addition of sulfur, as can be seen by a relative comparison. If the check plants are placed at 100, the following figures were secured:

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
1	100	6	100	11	100
2	100	7	150	12	100
3	60	8	110	13	90
4	30	9	80	14	70
5	5	10	0	15	60

Sulfur added in large quantities depressed the formation of nodules considerably. The phosphate together with sulfur seemed either to have no influence, or were rather depressing. This may be seen if the relative numbers of nodules produced on the roots of plants to which sulfur alone was given are compared with the nodule formation on roots of plants which received phosphate in addition.

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
2	100	7	80	12	90
3	100	8	70	13	90
4	100	9	100	14	60
5	100	10	0	15	100

This seems to point toward a certain degree of stimulation by small additions of sulfur, but depression when the acidity produced becomes too great.

EXPERIMENTS IN SAND CULTURES

The acidity produced by inoculated sulfur in the soil cultures seemed insufficient to attack the rock phosphate added.

To test out whether or not a similar phenomenon of sulfate production from inoculated sulfur in the presence of rock phosphate could be intensified whereby the phosphorus would become more rapidly available, and to determine the effect of this intensified process on plant growth, soy beans were grown in washed quartz sand, using Shive's nutrient solution (R^5C^2) as a basis.

Florida soft rock phosphate was substituted for the phosphorus of the nutrient solution. The rock phosphate and inoculated sulfur had been mixed about 6 weeks previous to the application, kept in tumblers in an incubator, and tested for accumulation of acidity and for formation of sulfates. From these tests it was apparent that sulfur oxidation had begun. The mixture was added at the rate of 2 tons per acre which supplied approximately the calculated amount of phosphorus in the check (Shive's R^5C^2 solution) cultures.

Hydrogen-ion concentration determinations were made at the beginning of the experiment and regularly at the end of each week during the period in which the plants were growing.

The results obtained are given in table 4.

The inoculated sulfur with the rock phosphate mixed previous to the application which was substituted for the phosphorus in the cultural solution, had, at the end of 6 weeks, produced a fair growth of these plants. In the cases where inoculated sulfur was added the high acidity had killed the plants after about 14 days. The soy bean plants grown in Shive's nutrient solution to which inoculated sulfur was addded made a good growth at first, but in 3 weeks the plants, which untill then had survived, died. The roots were dark brown, with the appearance of being burnt.

Sulfur oxidation went on rapidly in the cultures to which the inoculated sulfur rock phosphate mixtures were added, as is indicated by the lowering of the pH values. Adding inoculated sulfur only to Shive's nutrient solution

TABLE 4

Results of growing soy bean plants in sand cultures for 6 weeks with inoculated sulfur and rock phosphate, and Shive's R_5C_2 solution as a basis

CULTURE NUMBER	TREATMENT	WEIGHT OF TOPS	AVERAGE LENGTH	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION
		gm.	*cm.*		*pH*	*pH*
1	None	0.850	8	Bloom	5.8	6.8
3	Rock phosphate	1.454	11.5	Bloom	5.4	6.6
5	R_5C_2	3.610	15.0	4	5.3	6.6
6	R_5C_2, rock phosphate	2.223	15.0	2	5.0	6.4
8	R_5C_2, inoc. sulfur, rock phosphate	0.600	Dead		4.7	2.9
14	R_5C_2, inoc. sulfur	0.811	Dead		5.2	4.3

had also a decided influence on the lowering of the pH value, but no such great differences occurred as in the cultures to which previously mixed inoculated sulfur was added. This could be expected since sulfur oxidation had already begun in the mixture. Nevertheless, the plants grown in all cultures were killed in a comparatively short time. In the cases were no sulfur was added the plants made the soil solution less acid, bringing it nearly to the neutral point. This corresponds with the experiences in culture solutions where a similar phenomenon takes place. The changes in hydrogen-ion concentration were regular as is shown by the pH determinations, which were made at definite intervals.

Apparently not sufficient acidity was produced in the soil cultures, since the acidity at which the phosphorus of the rock phosphate becomes soluble had not yet been reached. In the sand cultures this point was reached, but the plants were not able to survive. The fact that very little or no phosphorus is changed into a soluble form, has been found in the studies reported in another paper of

this series. To find the exact acidity necessary to change the Florida soft rock phosphate used in these experiments into soluble P^2O^5, a curve was constructed from readings of pH values obtained by adding different amounts of 0.1 normal and normal sulfuric acid to the rock phosphate.

Ten grams of rock phosphate were shaken with definite amounts of sulfuric acid for 2 hours in a shaking machine and left standing overnight. An aliquot of the supernatant liquid was then drawn off and the hydrogen-ion

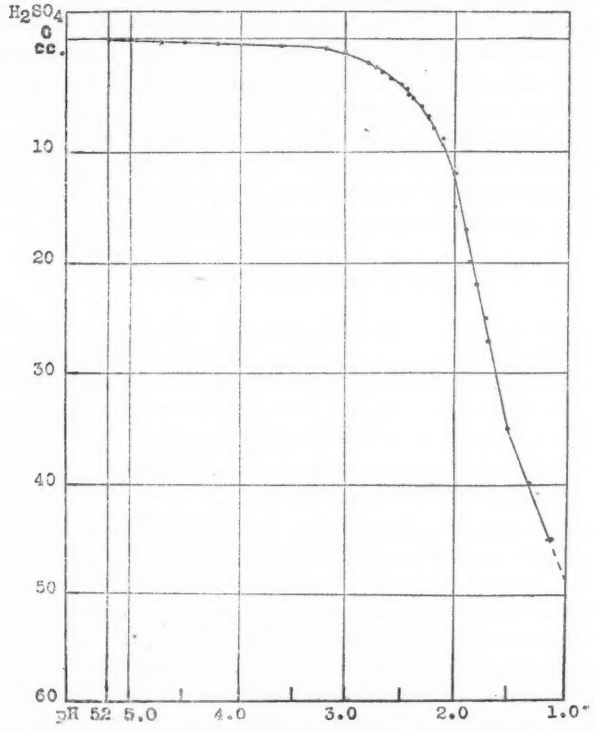

FIG. 1. GRAPH SHOWING THE HYDROGEN-ION CONCENTRATION AT WHICH ROCK PHOSPHATE BECOMES AVAILABLE

determinations made. At the critical point sufficient determinations were made to check up all points obtained. The results are graphically shown in figure 1.

The curve shows that the phosphorus of the rock phosphate becomes rapidly available when a point is reached between pH 3.1 to 2.8.

Since under ordinary circumstances not sufficient acid is produced in the soil it is very doubtful whether greater beneficial effects can be expected from a mixture of rock phosphate and sulfur, which have not been composted, previous

to the application, long enough so that most of the sulfur is transformed into sulfates and which has reacted with the phosphorus of the rock phosphate. It is very likely that the free acid produced by the sulfur oxidation is harmful to plant growth, more than the total amounts of sulfates or the so-called total acidity. Beneficial effects might be derived from inoculated sulfur which has not been composted with rock phosphate but mixed with the rock phosphate before applications are made, and when applied in small quantities, if the soil is in need of sulfur so that plant growth is stimulated. The plants under observation seemed to be stimulated by the sulfur to make more extensive root systems and thus would be able to take up greater amounts of plant food from the soil solution or from the slowly available rock phosphate present. At the same time the acidity produced would be not too intensive to be harmful to the normal development of roots and tops. It seems, however, more safe to compost the sulfur rock phosphate some time before application until most of the sulfur is converted into sulfates and has reacted with the phosphorus of the rock phosphate, in order to avoid the injurious effects.

BIOLOGICAL EXPERIMENTS WITH SOIL 1

In several instances it has been reported that sulfur and sulfates exerted a stimulating influence on bacterial growth. The possibly inadequate supply of sulfur in soils is apt to influence the microörganisms, and following the addition of certain quantities of it, a change in the biological flora can be expected.

The work reported in this part of the paper was mainly carried out to study the change in bacterial numbers and but slight attention was paid to the occurrence and determination of different species.

The amounts of sulfur added are given in table 5. During the incubation period hydrogen-ion concentration determinations of the soil water extract were made at frequent intervals. It was found that in all cases the hydrogen-ion concentration became greater until a certain point was reached, from then on the movement was back toward the neutral point. This turning point was reached in all cases before or between the ninth and the eleventh week of incubation. It should be remembered that no plants were growing in these tumblers. It seems, therefore, that the sulfates formed reacted with the soil constituents, bringing the soil solution back to a certain equilibrium. This phenomenon is even more clearly demonstrated by the curves shown in figure 2.

Bacterial numbers were determined by the plate method, using Lipman and Brown's synthetic agar, at the beginning of the experiment and after 6, 12 and 18 weeks. The numbers in thousands per gram of soil are given in table 5.

The bacterial numbers in the untreated soil remained about constant during the incubation period. The biological flora in the soils treated with amounts of sulfur up to 1500 pounds per acre seemed to be stimulated for the first 6 weeks, but the numbers in soils treated with greater quantities showed a rapid decline. The higher the applications of sulfur and consequently the higher the

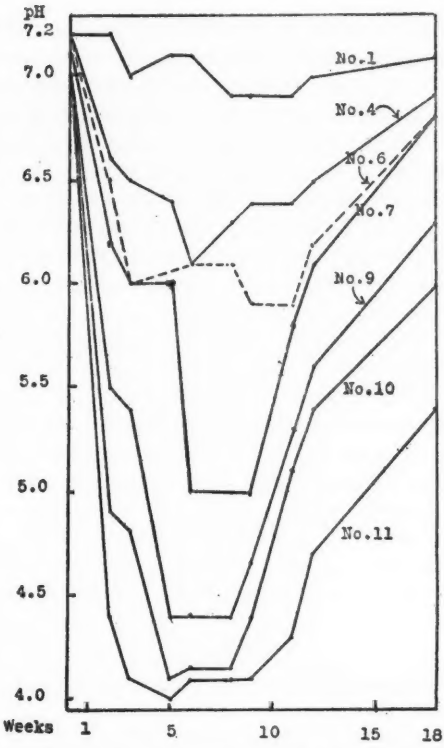

FIG. 2. CURVES SHOWING CHANGES OF HYDROGEN-ION CONCENTRATIONS OF SOIL 1 TREATED WITH DIFFERENT AMOUNTS OF INOCULATED SULFUR

TABLE 5

Bacterial numbers in soil 1 treated with inoculated sulfur

CULTURE NUMBER	SULFUR ADDED PER ACRE	NUMBER OF BACTERIA PER GRAM OF SOIL			
		Initial	After 6 weeks	After 12 weeks	After 18 weeks
	lbs.	*thousands*	*thousands*	*thousands*	*thousands*
1	None	3.280	3.195	3.200	3.150
2	100	3.240	2.520	3.525	3.475
3	300	3.190	3.470	3.615	3.535
4	500	3.200	4.470	3.325	3.225
5	1000	3.195	4.160	3.165	3.100
6	1500	3.260	3.600	2.720	2.950
7	2000	3.240	2.675	1.840	1.950
8	2250	3.275	2.390	1.415	0.850
9	2500	3.260	1.935	1.345	0.550
10	3000	3.235	1.405	0.765	0.450
11	3500	3.250	1.390	0.560	0.350

amounts of acidity produced, the more sulfates there were formed, the fewer colonies were counted on the agar plates. After 12 weeks much of the stimulating effect seemed to be lost in the cultures which had previously produced increased numbers. Applications of 100 and 300 pounds of sulfur to the acre seemed somewhat consistent in the production of higher numbers, but the decrease was very marked in the cultures receiving from 2000 pounds upward. The numbers in the cultures to which 3500 pounds of sulfur per acre was added had at the end of 12 weeks but slightly more than one-sixth of the original numbers. There is no doubt, however, that the depression of the original biological flora was accompanied by an extraordinary increase in sulfur-oxidizing organisms, but since these organisms do not reproduce on ordinary agar plated, or at least cannot be counted in the ordinary way on account of their extremely small size, no estimation of their numbers could be made.

After 18 weeks of incubation the amounts of sulfur oxidized were greater for all cultures which received the higher applications, but the movement of the hydrogen-ion concentration was still farther toward the neutral point. The numbers of colonies counted on agar plates increased in two cultures, while others showed a greater decrease. Cultures to which 3500 pounds of sulfur were added had at the end of 18 weeks but slightly more than one-tenth of the original numbers present.

After 24 weeks the majority of the cultures had reached the neutral point.

SUMMARY

Small amounts of inoculated sulfur had little or no influence upon the change of the hydrogen-ion concentration of soil 1, which had received fertilizers for about 30 years, but additions of larger quantities lowered the pH values.

The influence of sulfur oxidation was greater in soil 2, which had received no fertilizers for 33 years, and which was poorer in nitrogen and mineral plant food constituents than was soil 1.

Hydrogen-ion concentration increased nearly proportionally to the sulfur application.

Soils receiving rock phosphate in addition to the sulfur had usually higher hydrogen-ion concentrations than the soils in the pots receiving sulfur alone.

Soy bean plants, grown in soil treated with inoculated sulfur, were stimulated by small additions of sulfur, but were injured by larger additions.

Sulfur with acid phosphate in addition produced best soy bean plants, while the series receiving rock phosphate in addition were poorest.

The root systems of these soy bean plants were stimulated by small quantities of sulfur, but depressed by larger amounts.

Nodule formation seemed to be stimulated with small amounts of sulfur, but decreased numerically with the increase of the quantities of sulfur applied.

The phosphorus of Florida soft rock phosphate used in the experiments becomes available when a point in hydrogen-ion concentration between pH values 3.1 to 2.8 is reached.

The acidity produced by the oxidation of sulfur in these soils was not sufficient to render appreciable amounts of phosphorus more available, although the acidity produced was harmful to the soy-bean plants.

It is shown in sand cultures, that if sufficient acidity is produced to make the phosphorus available, the plants are killed.

Doubt is expressed whether greater beneficial effects can be expected from phosphate and inoculated sulfur mixtures, which have not been composted long enough previous to the application so that the sulfates and free acid formed have reacted with the phosphorus of the rock phosphate, than rock phosphate alone, unless the soil is in need of sulfur.

The hydrogen-ion concentration became greater in the uncropped soil with sulfur additions, until a certain point was reached; from then on the movement was back toward the neutral point.

The biological flora, expressed in numbers counted on agar plates from soil infusions, was slightly stimulated by small sulfur applications, but considerably depressed with larger amounts.

The formation of sulfates was influenced by the water content of the soil.

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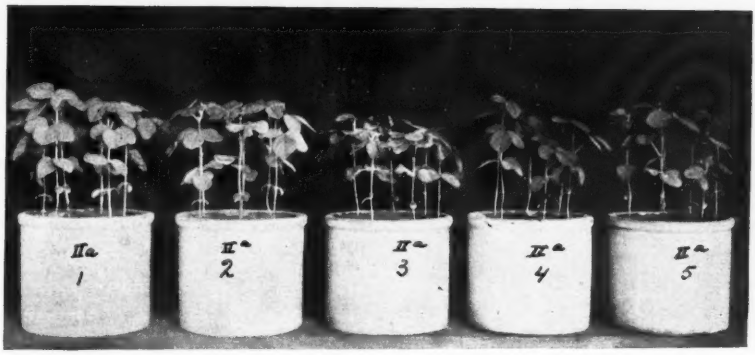

FIG. 1. SOY BEAN PLANTS GROWN IN SOIL WHICH HAD NOT RECEIVED FERTILIZERS FOR 30 YEARS. SULFUR ALONE ADDED. NO. 1 IS CHECK

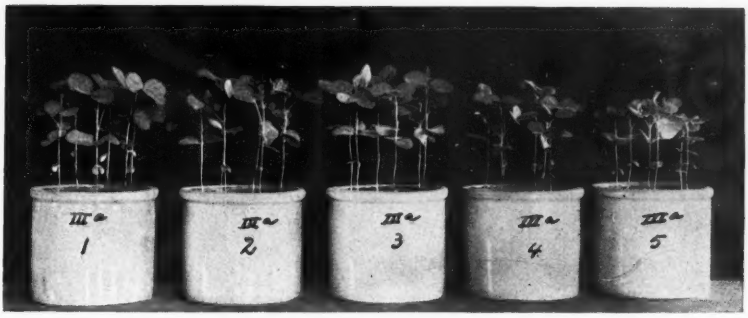

FIG. 2. SOY BEANS PLANTS GROWN IN SOIL WHICH HAD BEEN FERTILIZED FOR MORE THAN 30 YEARS TO WHICH SULFUR ALONE WAS ADDED. NO. 1 IS CHECK

CALCINED PHOSPHATIC LIMESTONE AS A FERTILIZER[1]

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INTRODUCTION

Before presenting experimental data it may be well to indicate the extent to which phosphatic limestone occurs, the quantities of phosphate held in it, and the significance which would attach to a method for profitable utilization of this source of phosphatic fertilizer.

RELATION BETWEEN PHOSPHATIC LIMESTONE AND PHOSPHATE ROCK

The brown phosphatic limestone from central Tennessee consists essentially of nodules of apatite varying in size from very fine dust to particles that will not pass a 20-mesh sieve, held together by a matrix of limestone, principally calcium carbonate. Where conditions have been favorable, the carbonates have been removed through solution in carbonated water and the much less soluble tricalcium phosphate is left but little affected except for the inevitable rounding off of sharp corners during the weathering process and except, also, for the deposition upon their surfaces of iron and aluminum oxides. This iron and aluminum existed in small amounts in the rock from which the phosphate has separated and the result of the weathering has been, through the removal of lime carbonate, a concentration, not only of the lime phosphate, but also of the iron and aluminum. Other substances, such as silica, which existed in the limestone and which are but slightly soluble in ground water remain mixed with the phosphate rock and reduce the tricalcium phosphate content to about 80 per cent or less. It sometimes happens, after the "brown rock" has been liberated from the limestone and concentrated, that it is more or less loosely bound together again by some cementing material which causes it to cohere in chunks of varying degrees of hardness when mined. These chunks will hold together well enough so that they can be built into piles over wood which is burned for the purpose of driving off moisture. Very much of the brown phosphate rock, however, exists as a loose granular deposit which forms a mud with water and which is easily removed from the ground with a shovel.

[1] Paper No. 43 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

Just why and how this phosphatic material came to be deposited with the limestone has not been definitely learned though it is supposed to have originated from the remains of some form or forms of marine life, but, since all known deposits of "brown rock" in central Tennessee have come from phosphatic limestone, these deposits, however extensive they may be, can exist only where conditions have been favorable for the dissolving action of ground water without the removal of phosphate material by erosion. Naturally, then, these deposits are limited in extent in comparison with the original limestone formation and, as a matter of fact, they are very limited in comparison with the still existing deposit of phosphatic limestone.

In many places the over burden of soil and rock has been so thick or so impervious, or has in other ways so protected the phosphatic limestone that it has not permitted this leaching. This condition still exists over extensive areas of the central basin of Tennessee and a study of any geological map of the area will show how extensive is this geological formation. Under this condition the phosphatic limestone may be covered by a thick layer of soil and rock or it may lie near the surface.

There are still other large areas where, during the most recent geological years, extending to the present time, erosion has been more than keeping pace with the removal of limestone by leaching. Where this erosion has not been so extensive as to remove the limestone itself in addition to what phosphate rock may have been liberated, the phosphatic limestone is left at or near the surface. A geological map shows that areas of this nature are also much more extensive than are the areas of workable deposits. It may reasonably be that the overlying strata have protected much of this limestone from leaching so that very little phosphate has been liberated. In this case the limestone has remained nearly or quite intact while it has continued to be uncovered by erosion.

In addition to the areas which exist under these two general conditions, there is also a considerable amount of phosphatic limestone which exists in or together with the workable deposits of phosphate rock. Rarely ever has the solution of limestone been complete. Even in those places where there are the best deposits of brown phosphate rock there exist unleached boulders and ledges of limestone termed "chimneys" or "horses" by the miners. Figure 1 shows the appearance of this limestone in a mine near Columbia, Tennessee after the phosphate rock had been removed.

The quantity and extent of the phosphatic limestone is many times greater than that of the phosphate rock. The quantity of phosphorus still locked up in the limestone in excess of that found in the rock phosphate deposits is roughly proportional to the excess of area still occupied by the phosphatic limestone over that occupied by the phosphate rock deposits.

Although new deposits of phosphate rock are still being found and opened up, the demand for the product has increased to such an extent that the methods of mining and purifying the phosphate have several times been improved to recover larger and larger percentages of the deposit.

PATENTS FOR THE UTILIZATION OF PHOSPHATIC LIMESTONE

Two patents (no. 971830 and no. 13302) aiming at the utilization of phosphatic limestone for fertilizer purposes have been taken out in this country. The plan of these patents is to make the phosphorus available to plants by burning phosphatic limestone at a temperature sufficiently high to drive off carbon dioxide and then slaking to break up the mass into a fine state of division. It has been claimed that this treatment, by breaking the phosphate nodules into a very fine state of division would make the phosphorus available.

More recently (2) a very similar German patent, no. 321776, has been granted. In this case a mixture of limestone and phosphate rock is burned.

Since limestone, heated to a temperature sufficient to drive off carbon dioxide slakes readily and reduces to a very fine powder, it remains only to determine how the phosphate in the phosphatic limestone is affected; to find out whether this too is pulverized, made porous or in any way made more available to plants.

During the summer of 1920 visits were made to a number of the worked phosphate deposits of central Tennessee and samples of phosphatic limestone were taken from several localities where there seemed to be an abundant supply of it easily accessible.

METHODS

In accordance with the method oulined in the American patents, quantities of these samples were broken into small pieces having a thickness of two inches or less and burned for ten hours at the full heat of a good gas furnace capable of holding six or eight pounds of the limestone when piled loosely with the muffle removed. The muffle was replaced by thin fire brick so that there was a reverbratory action of the flame. At the end of ten hours the lime was removed from the furnace and water was added to it while it was still hot and would slake readily. Only enough water was added to leave a comparatively dry powder when the slaking was complete.

This material, used in the tests reported below, was, for convenience, designated in the tables by the abbreviation HP and for brevity in discussions has been termed "hydrophos."

Under the treatment given, practically all of the carbon dioxide was driven off, as acid test showed, and the whole rock was reduced to what appeared from a superficial examination to be only hydrated lime. A sifting test showed, however, that a certain amount of it would not pass a 40-mesh sieve. One sample which had received the above treatment was rubbed well in a mortar with a rubber pestle. A sifting test of this showed about 12 per cent of the entire weight to be held on a 40-mesh sieve, 42 per cent on 100-mesh sieve, 18 per cent on a 200-mesh sieve and 28 per cent passing a 200-mesh sieve. Microscopical examination of the granules held on each of the sieves showed them to be coated with much finer particles of hydrated lime.

Another sample, after burning and slaking, was sifted and analyzed for the phosphorus content of the different separates. The results of the sieving and the analyses are shown in table 1 from which it may be seen that the composite sample after burning and slaking contained 23.5 per cent P_2O_5 equivalent to 51.35 per cent $Ca_3(PO_4)_2$.[2]

Microscopical examination in this case also showed hydrated lime adhering to all of the granules and yet the separates which did not pass a 100-mesh sieve contained more than one-half of the total phosphorus. The finer granules present a larger surface per unit weight to which hydrated lime may adhere.

TABLE 1

Sieving tests and phosphatic content of the separates from a sample of burned and hydrated phosphatic limestone

SIZE OF PARTICLES IN SEPARATES		WEIGHT OF SEPARATE		P_2O_5 IN SEPARATE	P_2O_5 AS FRACTION OF TOTAL WEIGHT	FRACTION OF TOTAL P_2O_5
Held by sieve	Passing sieve					
		gm.	*per cent of total*	*per cent*	*per cent*	*per cent*
40-mesh	20-mesh	25.9260	24.60	29.90	7.355	31.28
100-mesh	40-mesh	23.6708	22.46	29.20	6.558	27.89
200-mesh	100-mesh	13.3710	12.69	28.40	3.604	15.33
	200-mesh	42.4100	40.25	14.90	5.997	25.50
Totals.....		105.3778	100.00		23.514	100.00

TABLE 2

Analysis of granular separate from burned and hydrated phosphatic lime-stone held on a 200-mesh sieve and thoroughly washed to remove free lime

CONSTITUENT	CONTENT
	per cent
Moisture at 100°C.....	0.03
Total P_2O_5 [equivalent to 82.08 per cent $Ca_3(PO_4)_2$].....	37.60
Total CaO.....	52.00
Total K_2O.....	Trace
Iron oxides.....	0.40
Aluminum oxides.....	0.79
Silicon.....	Trace

This is probably sufficient to explain the fact that the finer separates contain a higher percentage of lime and a lower percentage of phosphoric acid. The separate passing the 200-mesh sieve, of course, contained a very large amount of hydrated lime not adhering to phosphatic granules. Evidently there has been little, if any, pulverizing of phosphate granules by the process of treatment, for the phosphoric acid passing a 200-mesh sieve was but one-fourth of the entire quantity, an amount which may well have existed as granules of this degree of fineness in the untreated limestone.

[2] Chemical analyses, unless otherwise stated, were made by Wiley & Co., commercial chemists located in Baltimore, Md.

A sample of the burned and hydrated product passing a 40-mesh sieve and held by a 200-mesh sieve, after thorough washing and repeated rubbing with a soft rubber pestle to remove as much of the fine lime as possible, when dried and pulverized to pass a 100-mesh sieve, showed an analysis of 37.6 per cent of P_2O_5 which is equivalent to 82.08 per cent of tricalcium phosphate. This product has been designated by the abbreviation TP ("thermophos") and was used in series A of the following tests. A partial analysis of it showed the results presented in table 2.

Microscopical and chemical examination of samples of burned and hydrated phosphatic limestone revealed no difference due to differences in the length of time of burning, so long as the temperature was high enough and maintained long enough to drive off all of the carbon dioxide.

CULTURE EXPERIMENTS

Chemical and microscopical tests all giving negative results on the value of the material, it remained for culture experiments to show what results might be expected from its field use.

Sand Cultures, Series A

It was thought that results from sand cultures might be influenced less by factors not under control. Accordingly 138 cultures were prepared in 1-gallon stone jars holding 5 kgm. quartz sand which had been washed with running water to remove fine material, rinsed with distilled water and finally air dried. This sand had a water holding capacity of 24.48 per cent of the weight of the dry sand and during the growing period of the cultures the moisture content of the sand was kept close to 60 per cent of its water holding capacity (733 cc. per jar) by frequently weighing and adding the necessary amount of distilled water. Six of the cultures were treated with sulfur without beneficial effect and are not reported here.

Soy beans of the Edna variety were transplanted to these jars, six plants to a culture on October 30, 1920. The number of plants per jar was reduced to three on November 30 and the final harvest was made on December 29. Weights were taken on the plants harvested November 30, but the results showed no significance which was not more strikingly shown in the results of the second harvest and, therefore, will not be reported.

On December 8, about six weeks after planting, solution was drawn from the bottom of each jar and its hydrogen-ion concentration determined by the colorimetric method. The pH values obtained varied from 5.0 to 8.5, but there was no relation between these values and yields or availability of the phosphate fertilizers.

With the following explanations the treatment of each of the cultures, as indicated in table 4 and the accompanying foot notes, will be easily understood. Except for the last 8 cultures (131–138), all the essential plant food

elements were added in the form of salts in solution. The salts used and the quantities of half molecular solutions per liter of the 733 cc. of moisture added to each jar at the beginning of the experiment were as shown in table 3.

The quantities of salts used and the volume of water added at the beginning were such as to make a solution having an osmotic pressuse value of about two atmospheres.

As a basis for the quantities of phosphorus to be applied, acid phosphate analyzing 17.8 per cent total P_2O_5 was applied in four different quantities equivalent to 200, 500, 1000 and 2000 pounds per 2,000,000 pounds of sand. Where phosphate fertilizers of other forms were used they were added in quantities which would supply the same amounts of phosphorus.

Burned and hydrated phosphatic limestone pulverized to pass a 100-mesh sieve contained 23.85 per cent of total P_2O_5 and 33.5 per cent of $Ca(OH)_2$ while the product obtained by sifting the unpulverized hydrated product through a 200-mesh sieve contained 13.76 per cent P_2O_5 and 51.29 per cent of $Ca(OH)_2$. For the purpose of comparison certain cultures without phosphorus and certain

TABLE 3

Nutrient salts and the amounts used in the sand cultures with soy beans

NUTRIENT SALTS USED	0.5 M SOLUTION PER LITER OF SOLUTION ADDED AT START	WEIGHT OF SALTS PER JAR
	cc.	*gm.*
$MgSO_4$	40.60	1.79
KNO_3	34.84	1.29
$Ca(NO_3)_2$	5.08	0.31

others with each of the forms of phosphate fertilizers other than the above were treated with hydrated lime in amounts equal to those contained in corresponding treatments with the above phosphate products. These facts are indicated in table 4 by the abbreviations HL^1 — HL^4 and HL^5 — HL^8.

Calcium cabonate in the form of a high grade limestone pulverized to pass a 100-mesh sieve and in amounts equal to those found in the four quantities of pulverized phosphatic limestone used, was added to some of the jars without posphorus and to some of the jars receiving each of the other forms of phosphatic fertilizers except burned phosphatic limestone ("HP").

As a check to see whether or not the burning of rock phosphate would cause it to have the same effect upon crop production that the burning might cause the phosphate in the limestone to have, a quantity of floats was burned for 10 hours at a temperature of about 800°–1000°C. and used in comparison with the other phosphates.

Since there seemed to be but little, if any, difference in yields when different quantities of the same phosphate materials have been used, yields for the treatments with the same materials have been averaged together as reported in table 4.

The probable error (1) of the relative yield values in tables 4 and 5 has been calculated in each case upon original weights of individual plants by the formula

$$\text{p.e.} = 0.6745 \frac{d^2}{n(n-1)}$$

The probable error of the percentage increases has been calculated in each case upon original weights by the formula for probable error of difference

$$\text{p.e.} = e^1 - e^2 \pm \sqrt{(a^1)^2 + (a^2)^2}$$

Symbols used in table 4

GL^1, GL^2, GL^3, GL^4 = ground limestone (88.41 per cent $CaCO_3$) at rates of 0.1708, 0.4270, 0.8540 and 1.7800 gm. per jar furnishing calcium carbonate equal to that added to the jars in PL^1, PL^2, PL^3, PL^4 respectively.

HL^1, HL^2, HL^3, HL^4 = hydrated lime (97.26 per cent $Ca(OH)_2$) at rates of 0.1402, 0.3506, 0.7011 and 1.4023 gm. per jar, supplying $Ca(OH)_2$ equal to that added in HP^1, HP^2, HP^3, HP^4.

HL^5, HL^6,HL^7, HL^8 = hydrated lime at rates of 0.5045, 1.2612, 2.5224 and 5.0449 grams per jar supplying $Ca(OH)_2$ slightly in excess of that added in HP^5, HP^6, HP^7, HP^8.

PL^1, PL^2, PL^3, PL^4 = phosphatic limestone (21.76 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

HP^1, HP^2, HP^3, HP^4 = burned and hydrated phosphatic limestone (23.85 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

HP^5, HP^6, HP^7, HP^8 = the fine separate passing a 200-mesh sieve from burned and hydrated phosphatic limestone (13.76 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

TP^1, TP^2, TP^3, TP^4 = phosphatic limestone, burned, hydrated, washed on a 200-mesh sieve, elutriated until nearly free from $Ca(OH)_2$, pulverized to pass an 100-mesh sieve (37.6 per cent total P_2O_5) and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

RP^1, RP^2, RP^3, RP^4 = floats (34.1 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

RP^5, RP^6, RP^7, RP^8 = floats passing a 200-mesh sieve (33.25 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

BRP^1, BRP^2, BRP^3, BRP^4 = floats (34.94 per cent total P_2O_5) pulverized to pass a 100-mesh sieve, burned for ten hours at about 800° to 1000°C. and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

AP^1, AP^2, AP^3, AP^4 = acid phosphate (17.8 per cent total P_2O_5) used at the rates of 200, 500, 1000 and 2000 pounds per 2,000,000 pounds of dry sand.

BS^1, BS^2, BS^3, BS^4 = basic slag (18.25 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

Results of Series A

From table 4 it may be observed that all the forms of phosphate produced some increase over growth obtained without phosphorus and without lime in some form, but none of the forms of phosphorus, except acid phosphate and basic slag, produced under any condition more growth than was obtained with ground limestone without phosphorus.

TABLE 4

Relative yields and probable error of soy beans in series A and percentage increase with probable error of difference for cultures treated with phosphorus over those without phosphorus

CULTURE NUMBERS	PHOSPHORUS TREATMENT	RELATIVE YIELD VALUES UNDER VARIOUS ADDITIONAL TREATMENTS†				
		No additional treatment	KN§	KN§ GL^1-GL^4	KN§ HL^1-HL^4	KN§ HL^5-HL^8
135–138	None	10 ±0.4				
1– 4	None		25 ±0.8			
5– 8	None			39 ±1.9		
9– 12	None				35 ±1.9	
13– 16	None					32 ±1.2
17– 20	PL^1-PL^4	(37 ±1.2)#	37 ±1.2			
Increase for PL^1-PL^4		*268%±13%**	*45%±6%*			
131–134	HP^1-HP^4	19 ±0.6				
22– 25	HP^1-HP^4		35 ±1.4		(35 ±1.4)#	
Increase for HP^1-HP^4		*95%±7%*	*41%±7%*		*3%±5%*	
26– 29	HP^5-HP^8		37 ±1.0			(37 ±1.0)#
Increase for HP^5-HP^8			*45%±5%*			*15%±5%*
31– 34	TP^1-TP^4		33 ±1.1			
36– 39	TP^1-TP^4			36 ±1.1		
41– 44	TP^1-TP^4				36 ±2.0	
45– 48	TP^1-TP^4					35 ±1.4
Increase for TP^1-TP^4			*30%±5%*	*−7%±6%*	*4%±7%*	*8%±6%*
49– 52	RP^1-RP^4		29 ±1.6			
54– 57	RP^1-RP^4			37 ±1.4		
59– 62	RP^1-RP^4				38 ±1.6	
63– 66	RP^1-RP^4					37 ±1.0
Increase for RP^1-RP^4			*16%±7%*	*−6%±6%*	*10%±6%*	*16%±5%*

67- 70	RP^5-RP^8		34 ±1.5			
71- 74	RP^5-RP^8			38 ±1.3		
75- 78	RP^5-RP^8				36 ±1.8	
79- 82	RP^5-RP^8					36 ±0.8
Increase for RP^5-RP^8			*35%±7%*	*−2%±6%*	*5%±6%*	*12%±5%*
83- 86	BRP^1-BRP^4		32 ±1.9			
87- 90	BRP^1-BRP^4			37 ±0.7		
91- 94	BRP^1-BRP^4				39 ±1.9	
95- 98	BRP^1-BRP^4					32 ±1.0
Increase for BPR^1-BRP^4			*27%±8%*	*−6%±5%*	*14%±6%*	*2%±5%*
99–102	AP^1-AP^4		78 ±3.6			
103–106	AP^1-AP^4			100 ±3.7‡		
107–110	AP^1-AP^4				95 ±3.4	
111–114	AP^1-AP^4					68 ±2.3
Increase for AP^1-AP^4			*209%±15%*	*156%±11%*	*175%±10%*	*115%±8%*
115–118	BS^1-BS^4		87 ±3.2			
119–122	BS^1-BS^4			76 ±1.8		
123–126	BS^1-BS^4				66 ±1.6	
127–130	BS^1-BS^4					53 ±2.6
Increase for BS^1-BS^4			*247%±13 %*	*95%±7%*	*91%±5%*	*66%±9%*

* Percentage increases with probable error of difference have been calculated on the actual weights taken rather than on the relative yields given in this table.

† All cultures were inoculated with a few cc. of a water-extract of soil from a soy bean field where nodule-forming bacteria were abundant, and were supplied with iron as the chloride and as the sulfide.

‡ Average weight of plant was 1.8968 gm.

§ KN is an abbreviation used to indicate a solution of salts supplying nitrogen, potassium, calcium, magnesium and sulfur as indicated in tabe 3.

Values in parentheses have been carried forward or back from "KN" column to the columns of other values with which they may properly be compared.

When used in addition to limestone or hydrated lime, neither "hydrophos" nor "thermophos" proved to be of more value than rock phosphate. The larger amounts of hydrated lime (HL^5 — HL^8) seem to have decreased plant growth, especially when used in addition to acid phosphate or basic slag. Cultures receiving acid phosphate except those to which the larger amounts of hydrated lime were added, produced two to four times as much growth as any other cultures except those treated with basic slag, while the corresponding cultures with basic slag produced one and two-thirds to two and one-third times as much growth as cultures treated with any other form of phosphorus except acid phosphate, comparing always those cultures which receive like treatment in addition to phosphorus. In no case with any of the phosphates used other than acid phosphate or basic slag, was the increase or decrease great enough or the probable error of difference small enough to indicate with any degree of certainty that the variation in yield from that of the corresponding culture without phosphorus was due to anything other than to individuality in plant growth.

The material prepared by simply burning and hydrating phosphatic limestone was not used with limestone sufficient to neutralize the soil acidity because it was thought that the phosphorus from this source would be more available in an acid soil. It is not probable that the difference in yield with and without limestone would have been greater than that for floats with and without limestone.

Sulfur inoculated with sulfur-oxidizing organisms, was added to six cultures with the hope that the acid produced would dissolve phosphorus and make it available. The amount added was 0.0522 gram per jar, equal to one-fifth the quantity of floats (RP^1) with which t was used in two of the cultures. None of the cultures so treated produced greater growth over the corresponding cultures without sulfur than may be accounted for by experimental error.

Soil Culture, Series B.

In addition to the sand cultures, another series of cultures was grown in soil. The soil was taken from the surface foot of a sassafras silt loam, from a field which had not been fertilized for 30 years or more and had not been under cultivation for a number of years. It contained 0.098 per cent of total P_2O_5 and had a lime requirement, according to the Veitch test, of 2000 pounds of CaO per 2,000,000 pounds of soil. Loss on ignition was 6.13 per cent and its hydrogen ion concentration at the beginning of the experiment corresponded to a pH value between 5.5 and 5.9. Its water holding capacity was found to be 46.86 per cent of the weight of the dry soil and during the growth of the cultures the soil was kept near 57 per cent of its water holding capacity. During the first part of the experiment the cultures were weighed when water was added, but towards the end they were watered without weighing.

Series B was soy beans of the Edna variety, four plants to a culture, grown from April 30 to July 2, 1921, in 2 gallon jars containing 7500 grams of air dry soil.

As in the sand culture series, acid phosphate was taken as the standard of phosphorus application, 400 pounds per 2,000,000 pounds of soil being the rate of application. Applications of other phosphate fertilizers were such that the amount of phosphorus added equaled that of the acid phosphate applications.

One culture was left without fertilizer treatment of any kind while the others received combinations of lime and a fertilizer mixture supplying nitrogen and potassium. Each of the cultures was inoculated with the proper legume bacteria. Details as to the kind and amounts of salts used and also other details of treatment are given in footnotes to table 5.

Symbols used in table 5

GL^1, GL^2, GL^4 = calcium carbonate (c.p.) used at rates of 1,000, 2,000 and 4,000 pounds of CaO per 2,000,000 pounds of soil respectively. The Veitch test showed 2,000 pounds of CaO to be necessary to neutralize the acidity of 2,000,000 pounds of the soil.

ML^1, ML^2 = magnesium carbonate (c.p.) used at rates equivalent to 1,000 and 2,000 pounds of CaO per 2,000,000 pounds of soil.

$(CaSO_4)^1$, $(CaSO_4)^2$ = calcium sulfate (c.p.) supplying Ca at rates equal to 1,000 and 2,000 pounds of CaO per 2,000,000 pounds of soil.

KN = potassium sulfate (c.p.) and sodium nitrate (c.p.) each used at the rate of 200 pounds per 2,000,000 pounds of soil.

K = potassium sulfate (c.p.) used at the rate of 200 pounds per 2,000,000 pounds of soil.

NO_3 = sodium nitrate (c.p.) used at the rate of 200 pounds per 2,000,000 pounds of soil.

NH_4 = ammonium sulfate (c.p.) used at the rate of 155 pounds per acre supplying nitrogen at a rate equivalent to 200 pounds of sodium nitrate per 2,000,000 pounds of soil.

HP = "hydrophos," phosphatic limestone burned ten hours, slaked with water while hot, pulverized to pass an 100-mesh sieve (23.85 per cent total P_2O_5) and used without further treatment at the rate of 298 pounds per acre supplying phosphorus at a rate equivalent to 400 pounds of acid phosphate per 2,000,000 pounds of soil.

AP = acid phosphate (17.8 per cent total P_2O_5) used at the rate of 400 pounds per 2,000,000 pounds of soil. Analysis showed the soil to contain .098 per cent total P205.

RP = floats (34.1 % total P_2O_5) pulverized to pass an 100-mesh sieve and used at the rate of 208 pounds (equivalent to 400 pounds of acid phosphate) per 2,000,000 pounds of soil.

OM = organic manure (fresh horse dung) used at the rate of ten tons per 2,000,000 pounds of soil.

KP = potassium acid phosphate ($KH_2(PO_4)_2$, c.p.) used at the rate of 3264 pounds per 2,000,000 pounds of soil.

CaP = calcium acid phosphate ($CaH_4(PO_4)_2$, c.p.) used at the rate of 2568 pounds per acre, supplying P_2O_5 at 91.45 % the rate of 3264 pounds of $KH_2PO_4)_2$ and supplying CaO at the rate of 615 pounds per acre, equivalent to 614 pounds of CaO per acre.

Results of Series B

The relative weights of dry tops for each treatment of series B are brought together in table 5 along with the probable error for each of them determined on the weights of individual plants. As indicated in table 5 these figures are in some cases averages of two cultures and in others they are of single cultures.

TABLE 5

Relative yields and probable error of soy beans in series B and percentages increase with probable error of difference for cultures treated with phosphorus over those without phosphorus*

CULTURE NUMBERS	pH VALUES OF SOIL AT HARVEST	ADDITIONAL TREATMENT†	PHOSPHORUS TREATMENT							
			None	HP		AP		RP		KP	CaP
				Yield	Gain*	Yield	Gain*	Yield	Gain*		
					per cent		*per cent*		*per cent*		
1, 11	5.7	None	17.8±2.3								
2, 12	6.8, 6.9	GL^4	16.1±0.7								
3, 4	6.8, 6.9	GL^4, NO_3, K	15.7±0.9								
5, 6	6.9	GL^4, NO_3, K		15.6±0.3	−1± 6						
7, 8	6.9, 6.8	GL^4, NO_3, K				20.7±0.6	32± 7				
9, 10	6.8, 6.9	GL^4, NO_3, K						16.3±0.5	4±6		
13, 14	6.7	GL^4, NH_4, K	16.5±0.8								
15, 16	6.8, 6.7	GL^4, NH_4, K		18.1±0.7	10± 7						
17, 18	6.7	GL^4, NH_4, K				24.5±1.5	49±11				
19, 20	6.7	GL^4, NH_4, K						16.7±1.3	1±9		
37	6.8	GL^2, ML^2, NO_3, K		38.4±0.4							
21	6.4	GL^2	29.7±2.3								
24	6.4	GL^2, NO_3, K	33.1±2.5								
25	6.6	GL^2, NO_3, K		35.5±3.5	7±13						
26	6.6	GL^2, NO_3, K				45.0±5.5	36±18				
27	6.4	GL^2, NO_3, K						32.5±2.6	−2±11		
22, 23	6.4	GL^2, OM	76.2±4.0								
33	6.3	ML^2	35.9±3.5								

34	6.0	ML[2], NO_3, K	42.0±6.8								
35	6.3	ML[2], NO_3, K		42.3±2.5	1±18						
36	6.0	ML[2], NO_3, K, $(CaSO_4)^2$		35.8±0.6							
42	6.5	GL[1], ML[1], NO_3, K		43.5±2.9							
28	6.1	GL[1]	41.4±2.5								
29	5.9	GL[1], NO_3, K	42.1±3.6								
30	5.9	GL[1], NO_3, K		47.6±6.9	13±18						
31	6.0	GL[1], NO_3, K				52.0±3.4	23±12				
32	6.0	GL[1], NO_3, K						40.3±2.4	−4±11		
38	6.0	ML[1],	45.4±3.3								
39	6.0	ML[1], NO_3, K	48.0±3.3								
49	5.9	ML[1], NO_3, K		48.3±3.0	1±10						
41	5.9	ML[2], NO_3, K, $(CaSO_4)^2$		48.6±4.7							
45	6.2	GL[2], NO_3								100.0±2.7‡	
43	5.8	NO_3								68.5±1.2	
44	5.7	NO_3, $(CaSO_4)^2$								67.7±2.2	
46	5.6	NO_3, K									60.5±8.8

* Percentage increases with probable error of difference have been calculated on the actual weights rather than on the relative yields given in this table.

† All cultures were inoculated with the proper nodule-forming bacteria.

‡ Average weight of plants was 14.568 gm.

Percentage increase or decrease with probable error of difference has also been calculated on weights of individual plants. The pH values included in this table were determined on samples of soil taken from each jar at about harvest time.

The response which this soil shows to heavy applications of phosphorus is indicated by the weight of culture 45, with 0.1623 per cent of KH_2PO_4, which produced the heaviest crop of all, and by the weights of cultures 43, 44 and 46, also with large applications of phosphorus, which produced the next heaviest yields, excepting only cultures 22 and 23 which were heavily fertilized with organic manure.

As table 5 shows, there are six possible comparisons between cultures without phosphorus fertilizer and cultures receiving "hydrophos." There are four similar comparisons possible for the effect of rock phosphate. In these ten comparisons the relation between percentage increase and the corresponding probable error of difference is such that in only one case is there so much as an even chance that either "hydrophos" or rock phosphate used at rates equivalent to 400 pounds of acid phosphate per acre produced an increase in growth. Acid phosphate produced increases large enough to be beyond question of doubt.

Barley Cultures, Series C

A third series of cultures consisted of barley grown in soil from the same source as that used in series B. Acid phosphate was used in amounts equivalent to 200 and to 400 pounds per 2,000,000 pounds of soil and both "hydrophos" and floats were used in corresponding amounts. The results obtained agreed with those from series A and series B in showing a decided increase in growth from acid phosphate, but no decided effect from either "hydrophos" or rock phosphate. Figure 3 shows a few cultures from this series the treatment of which varied only in phosphate fertilizer.

CONCLUSIONS

1. Burned and hydrated phosphatic limestone is considerably inferior to acid phosphate as a fertilizer either in sand cultures or in soil cultures.

2. The amount of hydrated lime supplied by using burned phosphatic limestone in amounts corresponding to 400 pounds of acid phosphate to the acre is entirely ineffective in correcting the lime requirement of an acid soil.

3. Any advantage that burned and hydrated phosphatic limestone may show over phosphate rock is so slight that it may be accounted for by the fact that its iron and aluminum content is lower and is in less intimate contact with the tricalcium phosphate.

4. Pulverized phosphatic limestone is as valuable for soil treatment as are any of the phosphatic products obtained in this test from the burning of this limestone.

REFERENCES

(1) MERRIMAN, MANSFIELD 1913 A Text Book on the Method of Least Squares. Wiley and Sons, New York.

(2) STOPPANI, E., AND VOLPATO, V. 1921 Disintegration of mineral phosphates for fertilizer manufacture. *In* Chem. Abs., v. 15, no. 10, p. 1595.

PLATE 1

FIG. 1. PHOSPHATIC LIMESTONE IN A PHOSPHATE ROCK MINE NEAR COLUMBIA, TENNESSEE, AFTER THE OVERLYING SOIL AND THE PHOSPHATE ROCK HAD BEEN REMOVED

FIG 2. SAND CULTURES FROM SERIES A

Culture 107 with acid phosphate.
Culture 25 with burned and hydrated phosphatic limestone ("HP").
Culture 44 with the washed granular phosphate from the burned and hydrated phosphatic limestone ("TP")
Culture 62 with 100-mesh floats
Culture 78 with 200-mesh floats
Culture 123 with basic slag

FIG. 3. BARLEY CULTURES IN SOIL FROM SERIES C—FERTILIZER SALTS (KN) AND ALSO THE PHOSPHATES ADDED TO THE SOIL FOR A PREVIOUS CROP OF SOY BEANS

Culture 38 without phosphorus
Culture 41 without phosphorus
Culture 6 with "hydrophos" (HP^2).
Culture 16 with acid phosphate (AP^2)
Culture 25 with floats (RP^2)
Culture 34 with c.p. tricalcium phosphate (PP^2).

FIG. 1

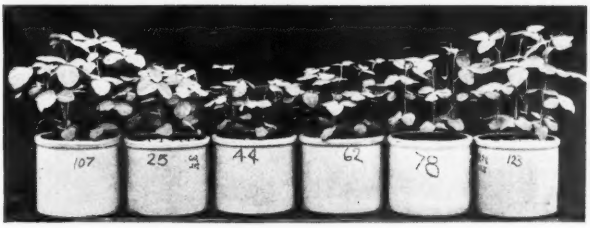

FIG. 2

FIG. 3

MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: II. METHODS OF THE STUDY OF NUMBERS OF MICROÖRGANISMS IN THE SOIL[1]

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INTRODUCTORY

In taking up the study of bacterial numbers in the soil as the first function for the characterization of the bacteriological condition of the soil, one should keep in mind that this is done not because it is the most important function, nor because it is known to be the most representative function, but for several other reasons. Following the determination of bacterial numbers in the soil by Koch in 1881 with the introduction of the gelatin plate, the first attempt was then made to make a thorough study of bacteria in the soil. With the possible exception of the decomposition of nitrogenous organic matter (ammonification) and nitrification, more work has been done in the study of numbers of bacteria in the soil as influenced by various factors, than of any other soil bacteriological function. This is a simple function, readily placed on a quantitative basis and does not have the complex qualitative character of the most other functions, which have not yet been placed fully on a quantitative basis. Our methods for determining bacterial numbers in the soil are well worked out, the limitations involved are well recognized and the variability factor can be readily calculated.

A historical review of the occurrence and distribution of bacterial numbers in the soil is found in the work of Voorhees and Lipman (17, p. 10–12), Löhnis (11) and various papers dealing with the subject Brown (2), Conn (4), Waksman (18), etc.

The determination of numbers of microörganisms in the soil has not been looked upon as of prime importance in the study of its bacteriological condition. The results obtained have been very variable, non-uniform and not very promising for the interpretation of soil fertility phenomena. A lack of confidence has been felt on the part of even the trained bacteriologist, who has recognized the limitations of the methods and found himself unable to correlate the results obtained by the determination of numbers of microörganisms

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in the soil with its crop producing power. This has led bacteriologists (Remy, 14) to state that the number of bacteria in any soil has but a very limited diagnostic value in ascertaining its fertility. This statement of Remy was based merely upon the fact that the number of colonies of microörganisms on the plate shows no direct relationship to the ammonifying, nitrifying or denitrifying power of the soil. It remains to be proved however, whether these last mentioned physiological activities are of diagnostic value. This untrustworthiness was also pointed out by Löhnis (10) who called attention to a difference in a small number of efficient bacteria and a large number of organisms possessing only a slight efficiency. He further points to the determination of bacteriological numbers in the soil as "rather worthless and spirit-destroying countings." In a later publication (12), Löhnis emphasizes this fact by stating that "all further investigations have shown, without any exception, that no definite relationship between the total number of bacteria and fertility of corresponding soil is recognized." Even as late as 1921, we find such a striking statement as "a quantitative bacteriological analysis of soil for total numbers of microörganisms has but a comparatively small significance as compared with that for the estimation of numbers in one or more physiological groups" (Northrup-Wyant, 13). Such a severe criticism of the value of determining the total numbers of microörganisms in the soil has been justified by the great variability in bacterial numbers reported by various soil bacteriologists and the lack of correlation between the numbers and the physiological activities of specific groups of microörganisms in the soil which are assumed to be of great importance to soil fertility (Remy, 14).

However, not all investigators have reached such negative conclusions as to the value of determining bacterial numbers in the soil. We may but refer to the work of Russell and Appleyard (16), who found the curves for bacterial numbers, nitrate content and carbon dioxide in the soil to be sufficiently similar to justify the view that all the phenomena are related.

The physiological activities of soil microörganisms will be taken up in the following papers, while here, we will limit ourselves only to numbers. A study of the variability and methods of mathematical interpretation of bacterial numbers has been reported in the previous paper (19) in this series. In this latter paper the author has endeavored to show that the variability and lack of correlation mentioned above are due to lack of uniformity in the methods, inaccurate methods, and changing soil flora. Nonuniformity of methods is made even worse by the fact that the details of the technic used by the various investigators are so widely divergent and that the data obtained even from one soil by the various methods are incomparable. Each investigator is, in the words of Northrup-Wyant (13), "a law unto himself in so far as the technic used in quantitative bacteriological soil analysis is concerned." Great variability in the numbers obtained on the same soil by the same investigator may also be due to the fact that the results are not checked sufficiently and the probable errors are too great for any accurate scientific work. This has been well recognized by various bacteriologists such as Chester (3).

METHODS

Although the methods used in a quantitative bacteriological analysis of soil have been reviewed in several of the more recent publications, as pointed out above, the various limitations of the methods have not been studied sufficiently, so that the ground is only incompletely covered. To determine just how much weight should be attached to the methods by which a particular soil phenomenon (function) is measured in comparison with the other soil microbiological activities (functions) in the building up of a system of soil bacteriology, a study of methods is of prime importance.

The various methods used for determining quantitatively the microörganisms in the soil can be classified into 3 groups:

1. Dilution method
2. Plate method
3. Direct counting method

The dilution method can be utilized not only for the determination of the total soil flora but also for the study of specific physiological groups, utilizing differential media. But the use of this method in routine bacteriological soil analysis is too cumbersome, involving a number of dilutions and cultures for each soil, and then only approximate results would be obtained, particularly in view of the fact that the selective action of the medium would be as manifest as in the case of the plate method, since not all organisms would develop on any one medium. The microscopic method suggested by Conn (5) gives promising results, but has not yet been developed to a sufficient extent to warrant any definite conclusions. The great difference between the plate and microscopic counts in normal soil is due to organisms which cannot be grown on plates. In the case of those organisms that develop on plates, the plate count will be nearly as high or even higher than the microscopic count, according to Conn. If we keep in mind, therefore, the limitations of the plate method, we will find it quite satisfactory for our work, until the direct counting method is developed sufficiently to warrant its exclusive use.

The plate method has been the one most commonly employed by bacteriologists, and has also been used in the present investigations. It is an indirect method, since we do not count the organisms directly, only the colonies that are produced on the plate. It is assumed that every bacterium, actinomyces, fungus spore or hypha develop into a colony. This can be justified only when the organisms are well separated from the soil into the diluting fluid, when the medium is favorable for the development of all these organisms, and when the temperature, oxygen supply and period of incubation are favorable. We can never obtain the ideal conditions but we can work out our technic so as to approach them as near as possible. The plate method has various limitations. The strict anaerobic microörganisms are excluded as well as the important groups of nitrifying, non-symbiotic nitrogen-fixing bacteria, sulfur-

oxidizing bacteria, to some extent the denitrifying, symbiotic nitrogen-fixing, pectin- and cellulose-decomposing bacteria. Then, of course, the algae and protozoa are eliminated. A further limitation is the fact that high dilutions are necessarily used, so that if groups of microörganisms are determined on the plate, those organisms occuring only in small numbers will give a rather inaccurate count. However, the fact that various important groups of soil microörganisms do not develop on the plate does not detract very much from the value of the method since it holds true for all soils, the microörganisms that do develop on the plate are constant soil forms, and no one method, with the possible exception of the direct microscopic method, will allow a study of all soil microörganisms. Therefore, the plate method within defined limits, will serve as a certain measure of the quantitative bacterial flora. The soil microorganisms that develop on the plate include the fungi, the actinomycetes and those bacteria which are concerned in the decomposition of organic matter in the soil, assimilation and certain transformations of minerals as well as other not sufficiently studied activities.

The composition of the medium is one of the most important factors in the determination of numbers of microörganisms in the soil by the plate method. It must be of definite chemical composition (synthetic) and must allow the development of the greatest number of microörganisms.

Both gelatin and agar media are usually employed in the plate method. Gelatin was the first solid medium suggested by Koch for the study of pathogenic bacteria and was also used for the study of soil bacteria by the same investigator. The advantages of agar media over the gelatin are several, chief among which is the fact that agar can be kept at higher temperatures than gelatin, that the gelatin-liquefying microörganisms do not interfere with accurate counts, particularly after a long incubation period which allows all microörganisms to develop. Agar media can be prepared of an exact chemical composition. This cannot be said of gelatin which is in itself a nutrient for various microörganisms. For qualitative work, gelatin media no doubt present certain advantages as in the case of the separation of bacteria into liquefying and non-liquefying groups. But even this separation is of doubtful value and may give different results under different conditions, since, under the best of conditions, it is a qualitative rather than a quantitative distinction. In the case of actinomycetes, for example, we find that they nearly all liquefy the gelatin, some in 3–4 days and some in 40–50 days.

Similar disadvantages are found in the case of the nutrient agar used chiefly by the earlier soil bacteriologists. The introduction of media of exact chemical composition (Lipman and Brown, 9 and Fischer, 6) was an important step in the standardization of the plate method.

A comparative study of the various synthetic media to be used for the determination of numbers of soil microörganisms, has been given by Brown (2) and by Conn (4). The medium used in the present work is a modification of Brown's albumen agar:

K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
Dextrose	10.0 gm.
Powdered egg-albumen	0.25 gm.
$Fe_2(SO_4)_3$	Trace
Agar	15.0 gm.
Distilled water	1000.0 cc.

All the ingredients, with the exception of the powdered egg-albumen are dissolved in boiling water, then filtered; the powdered egg-albumen is placed in a beaker and suspended by means of a stirring rod in a little distilled water, a drop of phenolphthalein is then added and sufficient 0.1 *N* NaOH is added from a burette until a distinct pink color is obtained. The egg-albumen is thus transformed into sodium albuminate, which is then added to the filtered agar and stirred in thoroughly. If the sodium albuminate solution has a few undissolved particles, it can be filtered first through a piece of filter paper. The use of sodium albuminate does away with the coagulation of albumen when added to the hot agar and allows the use of a standard product. The medium is tubed, in 10-cc. portions, and sterilized in the autoclave for 15 minutes at 15 lbs. pressure. The medium does not have to be freshly made up for each determination. The sterile tubed medium under cover for two months did not deteriorate so long as there was no appreciable drying out.

SOIL SAMPLING

It has been assumed that the same soil type, under the same conditions of treatment, contains at the same depth the same number of bacteria, and that soils differing in one way or another differ also in their bacterial activities (Hiltner and Störmer (8)). Due allowance must be made, however, to the natural variability of the soil itself. Where numerous samples are taken and thoroughly mixed, then carefully sampled, the danger is not so great as where only one or two samples are taken. By comparing the results obtained from various samples taken from the same uniform soil and treated alike, important variations have been obtained as pointed out in the previous paper of this series (19). For the actual field studies, four or five samples were taken from various parts of the plot, each sample being a composite of 3–4 borings, which were then thoroughly mixed.

Since the bacterial numbers are not the same at the various soil depths (18), the depth of sampling should be carefully considered. In taking samples for this study about $\frac{1}{4}$ inch of the surface was scraped away with a clean spatula and samples taken by means of a sampling tube to a depth of 6–6$\frac{1}{2}$ inches. The samples were taken into sterile containers, and counts made as soon as possible.

DILUTIONS AND PLATING

The first dilution was made as follows: 5 or 10 gm. or soil were added to a 250-cc. Erlenmeyer flask containing 100 cc. of sterile water; the flask was then

shaken for exactly 5 minutes; this gave a dilution of 1:10 or 1:20. Of the first dilution 1 cc. was then taken out, without allowing the soil to settle, and transferred into a flask with 99 cc. of sterile water, giving dilutions of 1:1000 or 1:2000. Higher dilutions than the last were obtained by adding 1 or more cc. of the last dilutions to corresponding amounts of water with each new dilution. The final dilution was such as to allow 30–200 colonies to develop on the plate. The flask is shaken for about 30 seconds. One cc. of the final dilution is then transferred by means of fresh, sterile 1-cc. pipettes into Petri dishes and the cooled agar is added. Eight to ten plates were used for each count. The plates were then incubated at 25°C. for various periods of time and all the colonies were counted with the naked eye.

The importance of using a comparatively large amount of soil to make the first dilution and making that dilution comparatively low, like 1:10 or 1:20, has been recognized by the earlier bacteriologists (Remy '14) as well as in the more recent investigations (Northrup-Wyant '13), since a more representative soil sample is thus obtained. Too large an amount of soil, as used in some cases, to give too low a dilution like 1:2, is also objectionable since a thorough mixture of the soil and water is difficult. It is entirely possible to obtain a thorough suspension of bacteria in water, since it has already been demonstrated by Hiltner and Störmer (8) that, on sufficient shaking, all bacteria are washed off the soil particles which remain almost sterile.

There is small need of calling attention to the importance of not allowing the soil to stand in contact with the water for more than the few minutes necessary for the manipulations of shaking, diluting and plating out. A longer period will lead to an appreciable decrease in numbers, due to plasmolysis of microörganisms as pointed out by Hiltner and Störmer (8). An increase in numbers may be obtained only in special instances, as in the case of frozen soil, air-dry soil and subsoil.

INCUBATION AND COUNTS

Various periods of incubation were used in the preliminary experiments, these finally led to the adoption of a 7-days incubation at 25°C. A shorter period of incubation will not allow a full development of all microörganisms and a proper differentiation between the bacteria and actinomycetes.

The numbers of microörganisms were estimated in the preliminary experiments on the basis of soil dried in a electric oven at 100°C. to constant weight. However this method of calculating the data is hardly logical. Microörganisms usually decrease in number with a decrease in the water-content below the optimum. In this connection the author does not fully agree with the more recent workers (Northrup-Wyant, 13) in this field who calculated the bacterial numbers only on the basis of a dried soil. In a soil which is almost dry, the numbers, which are small at that, are increased only very little by figuring back to an air-dry basis. In the same soil at a much higher moisture content, it is found that the addition of moisture did not serve merely

to dilute the soil, but had a stimulating effect upon the development of the bacteria, and it would hardly be advisable to multiply the numbers further to bring them to an arbitrary dry basis. The dry soil in itself does not signify anything, for the numbers depend on the relative moisture content as one of the important factors. Multiplying the numbers, to allow for the moisture, would be equivalent to doing the same calculation twice over.

To be scientifically accurate and have a basis for comparison, we should change the numbers in such a manner, by multiplying it by a moisture factor, so as to reduce those with a high moisture and increase those with a low moisture content. However, before such a factor has been found, the author agrees with Hiltner and Störmer (8) that the results should be reported per gram of moist soil (or even dry soil), giving also the moisture content of the particular soil as well as its optimum moisture (65–70 per cent of its water-holding capacity). The soil reaction, the author feels, should also be reported.

Perhaps, when our data are more complete, we may be able to calculate the potential bacterial activities from the soil type, its water content, reaction, nitrogen and carbon content, etc.

INFLUENCE OF MEDIUM

Preliminary work was carried out with the purpose of demonstrating the influence of the composition of the medium, reaction of medium, temperature of incubation, final dilution, etc., upon the numbers of microörganisms in the soil. The results are reported in tables 1–7.

Composition

Casein agar was prepared in a similar way to the egg-albumen agar except for replacing the egg-albumen by casein. Sodium asparaginate was made up according to directions given by Conn (4). Soil extract agar and urea nitrate agar were made according to directions given by Fred (7). The soil used for these preliminary studies was a greenhouse soil rich in organic matter, having an optimum moisture of 30 per cent and a reaction equivalent to pH 6.2. The fungi were not counted in these preliminary experiments, while under bacterial numbers, both bacteria and actinomycetes are included.

When the media of different composition are compared (table 1) the albumen agar, casein agar and soil extract agar are found to give the highest numbers. The last medium, although giving the largest numbers of all, has to be eliminated due to the fact that it is not standard in composition. The choice was then between the albumen and casein agar. The first was selected in spite of the fact that, in this experiment, it gave somewhat lower numbers than the casein agar. Albumen agar has been used by the writer for several years and has always given excellent results and stood out well in comparison with any other synthetic medium tested; it is also readily prepared and is of an exact chemical composition. Egg-albumen, of course, is not a pure protein,

but since it is used in the powdered form, it is always readily duplicated. This medium was, therefore, selected for further work.[2]

TABLE 1

*Influence of composition of medium on bacterial numbers**

PLATE NUMBER	ALBUMEN AGAR	CASEIN AGAR	SOIL EXTRACT AGAR	SODIUM ASPARAGINATE AGAR	UREA NITRATE AGAR
	colonies	*colonies*	*colonies*	*colonies*	*colonies*
1	86	107	109	78	62
2	106	119	134	72	43
3	93	96	103	67	54
4	78	103	128	94	57
5	84	114	113	66	56
6	102	133	136	76	48
7	85	112	142	83	38
8	94	101	131	87	47
9	74	122	98	69	55
10	89	109	118	72	53
Mean	89.1 ±2.23	111.6 ±2.33	121.2 ±3.22	76.4 ±1.91	51.3 ±1.52
σ	10.44±1.57	10.97±1.65	15.10±2.27	8.97±1.35	7.16±1.08
C. V.	11.7 ±1.7 %	9.6 ±1.5 %	12.5 ±1.9 %	11.9 ±1.8 %	14.0 ±2.1 %
Em	2.5 %	2.1 %	2.6 %	2.5 %	2.96%

* Plates incubated at 25°C. for 7 days; the figures designate the number of all the colonies on the plate except the fungi. Dilution 1:200,000.

Temperature and period of incubation

The data presented in table 2 show the influence of temperature and period of incubation upon the bacterial numbers found in a soil. When the plates are incubated at room temperature, practically no colonies developed in 2 days, while in 12 days not all the colonies seemed to have developed as yet, since more than twice as many colonies have been found in 12 days than in 5 days. By incubating the plates at 37°, not all the microörganisms are found to develop into colonies and the plates dry up on prolonged incubation. A temperature of 25 to 27°C. proved to be the most favorable, one or two degrees either way having little influence with a long period of incubation. The plates should certainly be incubated longer than even 5 days. Further experiments along this line have shown that, at 25°, there is very little increase in numbers

[2] After this study was completed, the author in coöperation with Dr. Fred of the University of Wisconsin (20) suggested, as definite uniform media for the determination of total numbers of microörganisms in the soil, a modification of the albumen agar given above and casein agar. The modification of the albumen agar consists in reducing the amount of dextrose from 10 to 1 gm. per liter, so as to prevent the development of spreading colonies. The casein agar is the same as the albumen agar, only 1 gm. of purified casein dissolved in 8 cc. of 0.1 *N* NaOH is substituted for the egg-albumen. However, the albumen agar used in the studies reported in this as well as in the following paper, was of the composition reported in the text above.

TABLE 2

*Influence of temperature and period of incubation on bacterial numbers**

PLATE NUMBER	ROOM TEMPERATURE			25°C.			37°C.		
	Incubation			Incubation			Incubation		
	2 days	5 days	12 days	2 days	5 days	12 days	2 days	5 days	12 days
	colonies	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*
1	No growth	76	152	38	120	164	28	102	Plates dried up
2		70	169	42	84	158	24	123	
3		58	128	35	105	172	16	126	
4		78	125	47	139	191	31	104	
5		56	130	48	118	197	26	134	
6		73	143	44	128	183	26	92	
7		53	88	43	126	152	21	79	
8		61	154	56	142	168	24	83	
9		64	134	39	81	179	18	67	
10		48	164	40	116	161	25	76	
Mean		63.7 ±2.18	138.7 ±6.0	43.2 ±1.28	115.9 ±4.39	172.5 ±3.14	23.9 ±0.9	98.6 ±4.93	
σ		10.22±2.28	23.47±3.55	6.01±1.34	20.65 ±3.11	14.72±2.21	4.26±0.95	23.14±5.17	
C. V.		16.0 ±3.6 %	17.6 ±2.5 %	13.9 ±3.1 %	17.8 ±2.7 %	8.5 %	17.8 ±4.0 %	23.5 ±5.2 %	
Em		3.42%	3.6 %	2.96%	3.79%	1.82%	3.77%	5.0 %	

* The numbers represent all the colonies on the plate, except the fungi. Dilution 1:100,000.

above 7 days, so that this period has been decided upon for future work. In this connection, attention should be called to the fact that Conn also found a seven-day period, at a temperature of 25°, sufficient since he seldom found any appreciable increase on further incubation.

The futility of short incubation periods (2–3 days), sometimes even at room temperature, is thereby made clear.

Reaction of medium

A slight acidity (+0.5−+0.25) has usually been recommended as the optimum reaction of the medium used for the bacteriological analysis of soils. Several portions of egg-albumen agar were adjusted to definite hydrogen-ion concentrations by means of 1.0*N* NaOH and 1.0*N* H_2SO_4 solutions and used for the plating out of bacterial numbers.

TABLE 3

*Influence of reaction of medium upon the growth of bacteria on the plate**

PLATE NUMBER	pH = 5.2	pH = 6.4	pH = 6.8	pH = 7.2	pH = 7.6
	colonies	*colonies*	*colonies*	*colonies*	*colonies*
1	62	77	63	53	44
2	56	105	66	66	42
3	52	83	91	41	38
4	89	69	79	64	34
5	86	76	87	56	41
6	47	97	83	45	43
7	96	92	88	58	45
8	51	89	97	51	39
9	57	86	61	44	24
10	69	78	76	48	36
Mean	66.5 ±3.76.	85.2 ±2.31	79.1 ±2.64	52.6 ±1.83	38.6 ±1.32
σ	17.68±2.66	10.87±1.64	12.41±1.87	8.6 ±1.29	6.22±0.94
C. V.	26.6 ±4.0 %	12.7 ±1.9 %	15.7 ±2.4 %	16.3 ±2.4 %	16.1 ±2.4 %
Em	5.65%	2.7 %	3.34%	3.5 %	3.4 %

* Dilution 1:200,000. Plates incubated 12 days at 25°. All colonies, exclusive of fungi, are reported.

The data presented in table 3 point definitely to the fact that a reaction of the medium equivalent to an hydrogen-ion concentration of about pH 6.4 is best. With more acid media, there is a decrease in bacterial numbers accompanied by a greater overgrowth of fungi. When the medium is made less acid there is also a drop in numbers, particularly above the neutral point, so that, at a pH 7.6, there are already less than a half as many colonies of bacteria developing than at pH 6.4. A reaction of about pH 6.5 is therefore, best. This happens to be the reaction of the egg-albumen agar when prepared according to the directions given above.

Method of preparing the dilutions

It seems to be generally agreed that the number of colonies to be allowed per plate should be between 30 and 200, for agar plates (Breed and Dotherrer, 1), or a narrower limit 50 to 150 for gelatin plates (Conn, 4). However, where the incubation is at 25° for 7 days, there is danger of fungi overgrowing the plates. A plate badly overgrown with fungi, particularly in the case of certain *Mucorales*, should be discarded and not considered in the final count. With the medium used, there is very little danger of bacterial spreaders.

The next two experiments deal with the method of preparing and time of shaking the soil with water in preparing the first dilution (table 4) and influence of the final dilution (table 5).

TABLE 4

*Influence of stirring and time of shaking upon bacterial numbers**

PLATE NUMBER	SOIL STIRRED IN MORTAR	SHAKEN 1 MINUTE	SHAKEN 5 MINUTES	SHAKEN 10 MINUTES
	colonies	*colonies*	*colonies*	*colonies*
1	144	104	158	144
2	168	114	168	168
3	170	125	162	171
4	153	136	184	155
5	156	113	162	147
6	178	122	181	158
7	149	131	151	175
8	153	116	174	168
9	171	119	189	167
10	158	108	179	151
Mean	160.0 ±2.3	118.8 ±2.11	170.8 ±2.65	160.4 ±2.31
σ	11.07±2.70	9.91±2.22	12.49±1.88	10.83±2.42
C. V.	6.9%	8.3%	7.3%	6.8%
Em	1.4%	1.77%	1.55%	1.44%

* Plates incubated 7 days at 25°C. Dilution 1:100,000. All colonies except fungi are reported.

The stirring referred to in the first column of table 4 was done by thoroughly stirring 5 gm. of soil in sterile water, in a sterile mortar for 5 minutes, pouring off the supernatant liquid into a sterile flask, stirring residue again for 2–3 minutes with a fresh portion of sterile water, and so forth, until only a few grains of pure sand remained. The shaking referred to in the other three columns was done by shaking 5 gm. of soil in 100 cc. of sterile water and withdrawing samples after 1, 5, and 10 minutes for the further dilutions. The final dilution for this experiment was 1:100,000. The results reported in table 5 were obtained by shaking the original dilution 5 minutes, and making the final dilutions 1 to 20,000, 50,000, 100,000, 200,000, 500,000 and 1,000,000.

The results obtained in table 4 justify, without further discussion, the conclusion that 5 minutes shaking is sufficient for suspending all the bacteria in

TABLE 5

*Influence of final dilution upon the bacterial numbers**

PLATE NUMBER	DILUTION, 1:20,000		DILUTION, 1:50,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	192	Too numerous to count	127	156
2	238		124	146
3	262		148	172
4	206		143	179
5	227		108	124
6	231		113	138
7	214		137	149
8	199		132	167
9	223		141	153
10	211		117	133
Mean	220.3 ±4.10		129.0 ±2.88	152.7 ±3.73
σ	19.25±2.93		13.51±2.03	17.53±2.64
C.V.	8.7 %		10.5 ±1.6 %	11.5 ±1.7 %
Em	1.86%		2.23%	2.44%
Average number of Bacteria per gm. of wet soil	4,403,000		6,450,000	7,635,000

PLATE NUMBER	DILUTION, 1:100,000		DILUTION, 1:200,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	76	113	29	59
2	65	106	28	62
3	56	103	30	61
4	74	112	27	69
5	62	142	38	73
6	59	135	24	64
7	67	129	33	68
8	51	127	31	56
9	63	118	41	61
10	69	131	36	74
Mean	64.2 ±1.64	121.6 ±2.81	31.7 ±1.12	64.7 ±1.28
σ	7.76±1.17	13.18±1.99	5.29±0.79	6.04±0.91
C. V.	12.1 ±1.8 %	10.8 ±1.5 %	16.6 ±2.5%	9.3 %
Em	2.55%	2.31%	3.53%	2.0 %
Average number of Bacteria per gm. of wet soil	6,420,000	12,160,000	6,340,000	12,940,000

* Numbers of all microörganisms, except fungi, are given.

TABLE 5—*Continued*

PLATE NUMBER	DILUTION, 1:500,000		DILUTION, 1:1,000,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	14	18	4	11
2	19	26	4	17
3	18	22	9	19
4	13	21	5	13
5	13	19	5	14
6	13	18	6	17
7	17	23	4	11
8	16	21	7	13
9	17	25	5	9
10	21	29	9	12
Mean	16.1 ±0.60	22.2 ±0.77	5.8 ±0.41	12.6 ±0.76
σ	2.81±0.42	3.62±0.55	1.93±0.29	3.53±0.53
C. V.	17.4 ±2.6 %	16.3 ±2.5 %	33.3 ±5.0%	28.0 ±4.2 %
Em	3.73%	3.47%	7.07%	6.03%
Average number of Bacteria per gm. of wet soil	8,050,000	11,100,000	5,800,000	12,600,000

the water. One minute is insufficient, while a period greater than 5 minutes proves to be injurious.

When the various dilutions are compared, it is found that both too low dilutions and too high dilutions give unfavorable results. With the low dilutions too many colonies develop on the plates and it is impossible to determine accurately even the number of colonies that have developed. With too many colonies on the plate, many microörganisms, particularly those that develop only late, fail to develop at all.

It is interesting to note that, while at 1:20,000 dilution the colonies were, at 7 days, so numerous that no accurate count could be made, particularly due to overgrowth of fungi and that with the 1:50,000 dilution, an accurate count was made, but the numbers of organisms obtained are much less than with the higher dilutions. This simply indicates that, with too many colonies on the plate, many organisms simply fail to develop. Another disadvantage of the too low dilutions is the fact that it is difficult to make an accurate differentiation, under these conditions, between bacterial and actinomyces colonies. Of course the advantage of the low dilution lies in the smaller error obtained, but this can be obviated by the use of a larger number of plates for the count.

In the case of too high dilutions, like those of 1:500,000 and 1:1,000,000, there is apt to be not only greater variability with a greater error involved and the actual elimination of many specific types but the actual count may be smaller.

In the case of the two highest dilutions used in this experiment, the number of colonies developing on the plate was below 30 and although in this case there was plenty of room for development, the count was less, even with a 7-days incubation period, than with the optimum dilution (1:100,000 and 1:200,000). This, chiefly, is the reason why the number of colonies allowed per plate has been usually recommended as between 40 and 200. The optimum dilution for ordinary field soils is from 1:100,000 to 1:200,000. For poor sandy soils, a lower dilution may have to be used; for heavily manured soils or green-house soils, higher dilutions should be used.

The results reported here are directly opposed to the claim of Rossi (15) that the number of microörganisms present in the soil depends entirely on the dilution and increases with the higher dilutions. That is true only within certain narrow limits (below the optimum dilution).

TABLE 6

*The use of tap water and salt solution (0.85 per cent NaCl) as diluents for making bacterial counts**

PLATE NUMBER	TAP WATER		SALT SOLUTION	
	All colonies except fungi	Actinomycetes	All colonies except fungi	Actinomycetes
	colonies	*colonies*	*colonies*	*colonies*
1	91	24	71	22
2	83	20	72	23
3	68	22	76	26
4	62	19	64	19
5	71	21	59	26
6	79	18	71	16
7	86	26	56	21
8	78	21	61	22
9	85	27	62	16
10	82	23	76	23
Mean	78.5 ±1.91	22.1 ±0.62	66.7 ±1.54	21.4 ±0.75
σ	8.98 ±1.35	2.92±0.63	7.25±1.09	2.53±0.53
C. V.	11.4 ±1.7	13.2 ±1.9 %	10.8 ±1.6 %	16.5 ±2.5
Em	2.43%	2.81%	2.31%	2.5%

* Dilution 1:200,000. Plates incubated 7 days at 25°C.

DILUENT

Ordinary sterile tap water is commonly used in making dilutions. The use of bouillon or sugar solution has not been found beneficial, while a solution of 0.4 per cent NaCl and 0.4 per cent KCl has actually been found injurious (Hiltner and Stormer, 8). Ordinary sterile tap water was compared with saline (0.85 per cent NaCl) solution with the results presented in table 6.

No advantage is obtained from the use of salt solution over ordinary tap water. If anything, there is an injurious effect due to the use of the salt solution, confirming the results of Hiltner and Störmer, who used a mixture

of sodium and potassium chlorides. No appreciable difference has been found in the number of actinomycetes with both diluents. The use of distilled water for making dilutions should be condemned, since plasmolysis will readily take place.

SUMMARY

With these preliminary experiments in mind, we can now establish some of the important points to be observed in the determination of bacterial numbers in the soil.

1. A medium of standard composition should be used, containing no peptone, meat extract, soil extract or similar material, which would vary greatly in composition. In addition to the necessary minerals and carbohydrate, the medium should contain a definite nitrogen source, like asparagine, purified casein or powdered egg-albumen.

2. The final reaction of the medium should be about pH 6.2–6.8, with an optimum at pH 6.5.

3. Sterile tap water should be used for making the dilution.

4. The soil should be shaken uniformly, for 5 minutes, in preparing the first dilution.

5. The original dilution should be 1:20 or 1:10, high enough to give a ready distribution of the bacteria, and low enough to allow a representative sample to be taken. The further dilutions should be uniform, preferably 1:10 or 1:100. The final dilution should be made in such a manner, as to give 40–200 colonies of microörganisms excluding fungi, per plate.

Where a count of soil fungi is wanted, special acid media should be used having a pH 4.0 (like raisin agar and special synthetic agar (Waksman, 19), which due to their nature, do not allow any development of actinomycetes or bacteria, so that a low dilution (one fiftieth to one two-hundredth of that used for bacteria) can be used. This, combined with a short incubation period will allow a count of fungi, involving a comparatively low probable error.

6. At least 3–5 samples, composite if possible, should be taken from each soil examined for each determination.

7. At least 6–10 plates should be used in plating out each sample. These last two points are important where we want to work out the variability of numbers of microörganisms, and reduce these to a mathematical standard.

8. The plates should be incubated at 25°C. for at least 7 days or at room temperature for at least 14 days, the first to be preferred due to uniform temperature.

9. Plates badly overgrown with fungi, particularly in case of certain *Mucorales*, should be discarded from the counts.

10. The numbers should be computed on the basis of wet soil or soil dried to constant weight, in each case stating the moisture-content and the moisture-holding capacity, or optimum moisture, of the particular soil.

11. The most probable error of the counts should not be greater than 2.0 to 2.5 per cent for each soil, and not above 3.0 per cent for each soil sample.

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NITRATE ACCUMULATION UNDER STRAW MULCH

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The surface application of straw has come into use quite widely by vegetable gardeners, orchardists, wheat growers, and others. Potatoes are often grown under a heavy mulch of four to eight inches of straw, and it is a general idea that mulching with wheat straw is good agricultural practice. Noticeable detrimental effects have suggested shortage of nitrogen so that along with other nitrate studies the writer has given attention to the effect of the straw mulch on nitrate production.

HISTORICAL

Previous to 1921 no study of this kind was found reported, but only recently there were reported results from a similar study by Scott (4) where the surface applications of straw on wheat as low as 2 tons per acre had depressive effects on the nitrate accumulation in the soil.

Laboratory studies have been common (1, 5, 7), showing detrimental effects on nitrification by organic matter in solution, but the mixture of organic materials through the soil (3) has not been so destructive.[1] The effect of the straw mulch as a surface dressing on nitrate accumulation remained to be tested.

EXPERIMENTAL

The following study was conducted from 1917 to 1919 inclusive, on a brown silt loam of glacial origin with fine friable structure to a depth of about 8 inches. Below this point it grades quickly into a rather tight, brownish mottled, silty, clay loam. The surface sloped eastward with a 3-per cent grade and the plots were surrounded by board curbing to prevent cross washing, so that the plots served as independent though adjacent units. Four plots, 1/80 acre each, were treated as follows:

PLOT NUMBER	SPRING TREATMENT	SUMMER TREATMENT
3	Plowed	Fallow, Straw mulch 8 tons per acre, weeds pulled
4	Plowed	Fallow, surface scraped
5	Unplowed	Fallow, surface scraped
7	Plowed	Fallow, surface cultivated

[1] Confirmed by Dr. J. G. Lipman, N. J. Agr. Exp. Sta., in recent correspondence.

The spring plowing was eight inches deep and the surface scraping of plots 4 and 5 was done by means of a hoe to cut off small weeds. The hoe was used to cultivate plot 7 to a depth of 2–3 inches after rains. The straw mulch was applied at the rate of 8 tons after plowing. In the following spring the remaining straw was removed, the ground plowed as soon as dry enough, and remulched with fresh wheat straw. Few weeds came through the mulch and little care was needed to keep them down. At the start, the land was cleared of a thin wheat crop and uniform conditions for all plots were represented.

Samples of the surface 7 inches of soil were taken every three weeks except when the soil was frozen, which occurred only in the winter of 1917–18. The open winters of the remaining two years caused little irregularity in this respect. Nine samples per plot were drawn in schematic order to form a composite sample. Only the surface soil data are reported because of the nature of the subsoil in this case and the lack of differences in nitrates at greater depth as shown by sub-surface samplings.

As soon as possible after sampling, the soil was mixed, 100-gm. portions taken, dried at 108°C. for 8 to 10 hours, and loss of moisture determined. These were then extracted with 300 cc. of 1/16 *N* hydrochloric acid. The nitrates were determined in 200 cc. aliquots by boiling down, reducing with DeVarda's metal, and distilling into standard sulfuric acid. Calculations are given in pounds of nitrogen as nitrate per 2,000,000 pounds of oven dried soil.

Table 1 gives the data on nitrate nitrogen in the plots during the time studied, while figures 1 and 2 give these data graphically.

From the data and graphs it is evident that the straw mulch prohibits the accumulation of nitrates in the soil. During the three years of study the nitrate nitrogen under mulch was never greater than 27 pounds per two million of soil,—reached in late July of the excessively hot and dry summer of 1918,—while in plot 4, without the mulch, this figure went over 200. Comparatively few of the minimum determinations of the unmulched plots went below even the maximum on the mulched plot, and the graph of the mulched plot meets that of any other plot for only two dates during the three years.

As to the cause of this failure of nitrates to accumulate, one must consider the moisture content of the soil. Table 2 gives the nitrates of plot 3 together with the percentage moisture at the corresponding samplings. Figure 3 shows this relation graphically. From the curves it is evident that there is a close reciprocal relation of moisture to nitrates. This relation points out that moisture was an inhibiting factor, for only as the moisture was lessened did nitrates accumulate. Evidently moisture is the main cause, directly or indirectly, for the inhibition of nitrate accumulation. The mulch serves to increase the moisture in the soil by increasing absorption through lessened run off and also by preventing evaporation.

One might be led to believe that nitrates might be formed but removed by the excessive water caught by the mulch. Were such true, the production of

TABLE 1

Nitrate nitrogen in mulched soil as compared with unmulched

(In 2,000,000 lbs. of soil)

1917					1918					1919				
Date sampled	3*	4	5	7	Date sampled	3	4	5	7	Date sampled	3	4	5	7
	lbs.	*lbs.*	*lbs.*	*lbs.*		*lbs.*	*lbs.*	*lbs.*	*lbs.*		*lbs.*	*lbs.*	*lbs.*	*lbs.*
May 12	9	13	21	14	Mar. 1	13	155	80	76	Jan. 27	8	95	39	24
June 4	0	26	29	24	Mar. 22	19	158	86	86	Feb. 19	10	90	79	27
June 26	5	62	46	41	Apr. 12	17	177	89	80	Mar. 12	8	96	63	15
July 19	14	106	79	61	May 3	11	63	26	21	Apr. 4	12	119	118	38
Aug. 9	9	97	98	75	May 24	5	82	47	25	Apr. 25	14	115	105	35
Aug. 30	7	103	88	54	June 14	24	208	104	82	May 23	5	118	100	18
Sept. 22	14	171	124	88	July 5	16	131	85	74	June 7	12	111	89	9
Oct. 13	10	189	125	65	July 26	27	124	29	31	July 2	8	98	107	
Nov. 3	5	194	140	84	Aug. 16	20	166	94	78	July 24	12	159	204	42
Nov. 24	17	205	171	104	Sept. 6	12	157	63	23	Aug. 8	16	81	265	43
					Sept. 27	9	238	126	76	Sept. 1	8	174	178	36
					Oct. 19	20	72	111	64	Sept. 22	13	146	213	24
					Nov. 8	7	126	167	55	Oct. 13	13	143	253	22
					Nov. 29	12	114	70	27	Nov. 3	10	112	177	17
					Dec. 21	9	98	60	17	Nov. 21	17	89	241	21

* Plot 3. Straw mulch.
Plot 4. Spring plowed; scraped.
Plot 5. Unplowed; scraped.
Plot 7. Spring plowed; cultivated.

TABLE 2

Nitrate nitrogen and per cent moisture under mulched soil (Plot 3)

1917			1918			1919		
Date	Nitrate	Moisture	Date	Nitrate	Moisture	Date	Nitrate	Moisture
	*lbs.**	*per cent†*		*lbs.*	*per cent*		*lbs.*	*per cent*
May 12	9	20.6	Mar. 1	13	22.5	Jan. 27	8	23.3
June 4	0	25.2	Mar. 22	19	23.4	Feb. 19	10	23.4
June 26	5	23.9	Apr. 12	18	22.2	Mar. 12	8	23.2
July 19	14	21.3	May 3	11	23.7	Apr. 4	12	22.8
Aug. 9	9	22.7	May 24	5	25.0	Apr. 25	14	20.1
Aug. 30	7	22.7	June 14	24	20.5	May 23	5	24.7
Sept. 22	14	20.0	July 5	16	22.4	June 7	12	24.1
Oct. 13	10	21.4	July 26	27	18.2	July 2	8	
Nov. 3	5	21.8	Aug. 16	20	20.9	July 24	12	20.1
Nov. 24	17	19.0	Sept. 6	12	23.5	Aug. 8	16	20.6
			Sept. 27	9	22.7	Sept. 1	8	22.3
			Oct. 19	20	25.0	Sept. 22	13	24.1
			Nov. 8	7	24.8	Oct. 13	13	22.9
			Nov. 29	12	24.7	Nov. 3	10	19.1
			Dec. 21	8	24.4	Nov. 21	17	14.2

* Nitrate nitrogen is given as pounds per two million soil.
† Moisture is expressed as per cent of oven-dry soil.

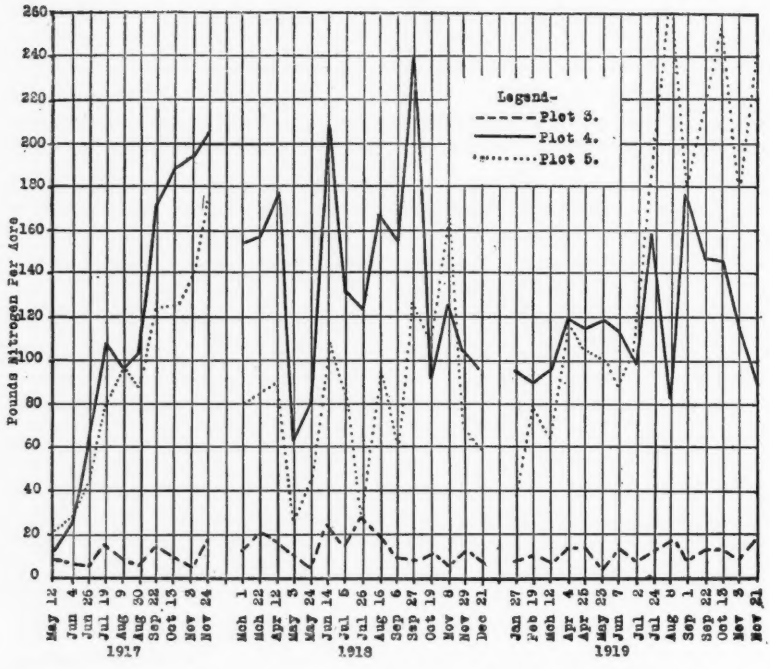

FIG. 1. NITRATE-NITROGEN IN SOIL UNDER MULCH (PLOT 3) AS COMPARED WITH UNMULCHED SOILS (PLOT 4, SPRING PLOWED AND SCRAPED; PLOT 5, UNPLOWED AND SCRAPED)

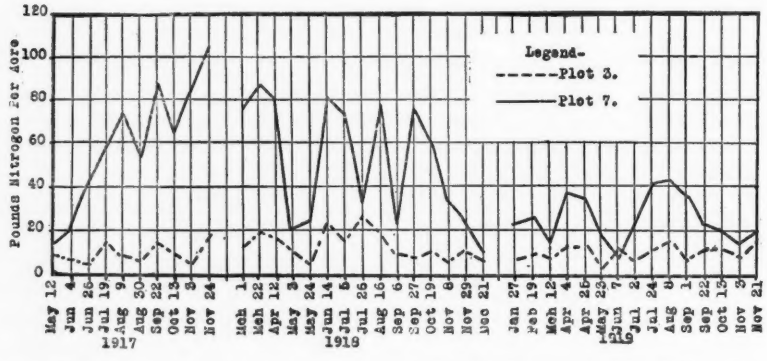

FIG. 2. NITRATE-NITROGEN IN SOIL UNDER MULCH (PLOT 3) AS COMPARED WITH UNMULCHED SOIL (PLOT 7,—SPRING PLOWED AND CULTIVATED THREE SEASONS)

nitrates after rain should give wider fluctuations than 19 pounds of nitrogen, which was the maximum increase found during any 3-week period of no rainfall following much rain. During the corresponding time, plots 4, 5, and 7 (with no mulch) showed increases of 126, 67, and 47 pounds respectively. Examination of any other 3-weeks period with no rainfall to remove the nitrate fails to show significant increase in nitrate content under the mulch, while in the other plots it shows great increases and establishes the certainty that nitrates are not accumulating at a rate comparable to that in soils without mulch. Had significant nitrate accumulation gone on in the surface and had the nitrates been carried downward, these could have been detected in the subsurface of heavy clay soil. Determination of nitrates on five inches of soil below the surface layer showed nitrates in constant amounts too in-

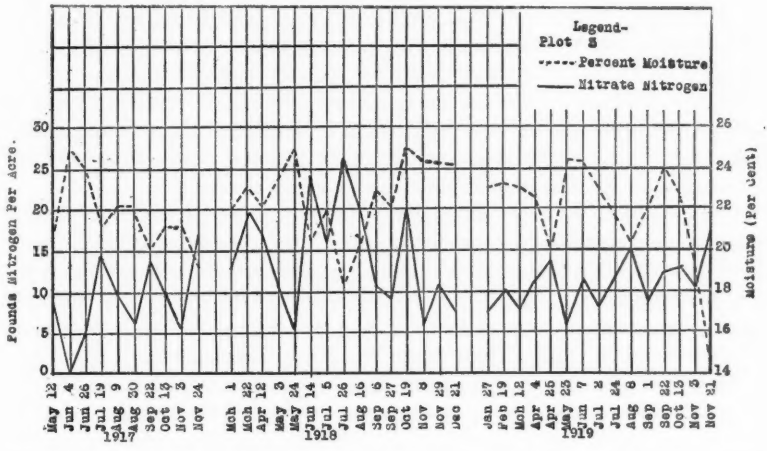

FIG. 3. COMPARISON OF NITRATE-NITROGEN WITH PERCENTAGE MOISTURE IN SOIL UNDER MULCH (PLOT 3)

significant to indicate sudden nitrate movement or important disturbances. No more than 12 pounds of nitrogen per 2,000,000 of soil were ever found in this layer.

According to Gainey and Metzler (2), the optimum moisture for nitrate production in a soil with any degree of compactness, will be reached when it contains approximately two-thirds of the total amount of moisture it will retain. Tests on this soil by the Hilgard cup method gave 40.23 as its moisture-holding capacity and according to the above, the optimum should be 26.82 per cent. Instead, there was no accumulation at 24–25 per cent, which was the maximum water content found in this soil. This suggests that possibly Gainey's figure is too high or the moisture is not the sole, direct, contributing cause of the failure of nitrates to accumulate. For that reason

daily temperature records were taken at a depth of 3 inches below the surface of the soil on the mulched plot, the unplowed plots and the plowed-scraped plot during the summer of 1918.

During June, July and August the average temperature of the mulched soil at 5.30 p.m. was 25.35°C. while on the plowed-scraped soil it was 33.06°C. and in the unplowed 33.92°C. The maxima were 30°, 40° and 39.5°C. in the order given above, showing that the mulched soil failed by ten degrees centigrade of being as warm as the plots not mulched. However, a temperature of 25.35°C. (the average for plot 3) should be high enough to encourage significant bacterial action so that higher nitrate production could be expected were temperature the sole limiting factor.

The lower temperature, of course, is due to the moisture of which the mulched soil always contained large amounts, and only in dry seasons did it dry out enough to put the soil in good tilth, or at about 50 per cent of moisture-holding capacity. By using this moisture figure for tilth rather than Gainey's for nitrification, the optimum for this soil would be 20.1 per cent or considerably below 22.4, the average water content of all the samplings for this soil. At this rate the soil was wetter than optimum by 2.3 per cent during most of the time under study and moisture may be closely connected in causal relation with the suppression of nitrate accumulation.

This inhibition of nitrate accumulation by the mulch is of particular significance to the vegetable gardner, horticulturist, wheat grower and others who have been using the straw mulch. Since crops require nitrogen and use it to best advantage in the nitrate form, it is possible that the low concentration of nitrates present at any time under the straw may be an inhibiting factor to best growth of certain crops. Since wheat is a heavy nitrate feeder it is readily possible that some of the detrimental results from mulching wheat have come from this cause as reported by Scott (4) even with as light an application as 2 tons per acre. Mulching with straw raises the moisture supply, but evidently does not allow nitrate accumulation to the concentration to be expected. Whether the process of nitrification goes on regularly and the nitrates are removed or changed by some other biological process encouraged through the presence of soluble organic matter,—as is the opinion of Lipman[1]—is a question which can be answered only by further study and analytical work.

Results obtained in this study suggest that mulching with straw keeps down the concentration of nitrates in the soil, and one may readily expect only those crops to do well which are able to obtain their nitrogen from this low a concentration. The nitrate content of soils under mulch is far enough below those under crops in similar studies at Missouri, or reported by Whiting (6), to suggest that mulching with straw is a practice to be used with care and discretion rather than one of universal application.

[1] Confirmed by Dr. J. G. Lipman, N. J. Agr. Exp. Sta., in recent correspondence.

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